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SPECIAL LISTING

CURRENT CANCER RESEARCH

on

GENETICS AND MOLECULAR BIOLOGY OF DNA TUMOR VIRUSES

JANUARY, 1981

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service ● National Institutes of Health ●
National Cancer Institute

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INTERNATIONAL CANCER RESEARCH DATA BANK (ICRDB)

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The present series of **SPECIAL LISTINGS** is a third edition, and begins with number 201. This series will update the second edition which included **SPECIAL LISTINGS** numbered 101 through 147. Depending on the total number and types of cancer research summaries received, the third edition will have at least 47 titles, and possibly a few more. In most cases, each third edition **SPECIAL LISTING** will replace the exact subject area of a second edition **LISTING** (SL 201 replacing SL 101, etc.). Whenever the number and variety of summaries requires a change in this procedure, a notation will be made in the specific **LISTINGS** affected.

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CURRENT CANCER RESEARCH

I. HERPESVIRUSES

A. HERPES SIMPLEX VIRUS

1. STRUCTURE AND GENOMIC ORGANIZATION

1. STRUCTURE OF DEFECTIVE HERPES VIRUS TYPE 1 GENOMES

Thompson, K. D., Enquist, L. W., Vande Woude, G. F., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Virus Tumor Biochemistry Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Fine structure restriction endonuclease maps of three classes of herpes simplex virus type 1 (HSV-1) defective DNA (dDNA) fragments cloned in lambda WES-gt B (ZO1-CP-04882-05-LMV and ZO1-CP-04914-02-LMV) have been determined. The major class, 9,000-10,000 base pairs, originates from the rightmost end of the HSV-1 genome. One of two minor classes of dDNA (6000-8000 b.p.) contains a site-specific 2000 b.p. deletion approximately 3000 b.p. from a region in dDNA representing the rightmost end in HSV-1. The other consists of at least two possible types of dDNA, those which contain 2000 b.p. less of HSV-1 of the rightmost DNA represented in the major dDNA class and a less well-understood type that appears to have internal rearrangements when compared to other members of this class and to the major dDNA class.

2. STATUS OF VIRUS DNA IN HERPES-TRANSFORMED CELLS

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OBJECTIVE: To find whether the HSV type 2 genome or part of it can exist as a stable genetic character in mammalian cells and to investigate the relationship of the virus DNA with the host chromosomes.

APPROACH: To introduce HSV-2 DNA fragments of various sizes into L-cells lacking thymidine kinase (LTK negative cells) and select cells which carry the HSV-2 thymidine kinase gene. These cell lines contain a small number of copies (2-4) per cell of a fragment of the HSV-2 genome lying within map units 0.2-0.4, and attempts to transform cells to a thymidine kinase positive phenotype with restriction digests of HSV-2 DNA show that the gene lies at a map position of approximately 0.3 and is therefore co-linear with the HSV-1 gene.

The quantity of virus specific DNA and virus specific enzyme is characteristic of each cell line and is stable to passage either in selective or non-selective medium. Several cell lines carry virus specific characters in addition to the kinase gene. These characters have been detected by the ability of the cell lines to compensate the defects in virus mutants, and these characters, like the TK gene, are also stable to passage.

Revertant cell lines have been obtained by selection in the thymidine analogues BUDR or acycloguanosine. These cells, which lack detectable thymidine kinase, fall into two classes: 1) those which contain no detectable virus specific DNA sequences, and which cannot revert to a TK positive phenotype - deletion mutants; 2) those which contain virus specific DNA and which can be induced to synthesise thymidine kinase by infection with a TK negative virus mutant.

3. HERPES VIRUS CODED INFORMATION IN CELLS DERIVED FROM CERVICAL CARCINOMA, PREMALIGNANT LESIONS OF THE CERVIX AND CELLS TRANSFORMED IN VITRO

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OBJECTIVE: To analyze which (if any) regions of the herpes simplex virus (HSV) genome are expressed in cells obtained from biopsies of cervical malignancy, premalignant dysplasia and carcinoma in situ, and from HSV transformed cells, and to relate this to the known physical maps of HSV coded functions and to other virogenes. The results may help resolve whether herpes viruses (or other viruses) play a role in the development of the disease.

APPROACH: Punch biopsy samples taken from patients with premalignant or malignant disease will be cultured in vitro, used to prepare thin sections and used as a source of nucleic acid extracts. An essential control will be to analyze the tissue for the presence of infectious virus. The cells and tissues will be examined for the presence of HSV coded RNA and DNA, using as probes highly specific HSV DNA obtained by cloning subfractions of the genome in the bacterial plasmid pBR322 (U.K. guidelines, GMAG proposal S607/21/2). The main approaches will be to test for m-RNA in specific cell types using in situ hybridization and to examine the total tissue content of virus coded nucleic acid using hybridization to virus or cell DNA immobilized on nitrocellulose membrane filters. Well characterized HSV-transformed cells will be used to calibrate the sensitivity of the technique. The approach has the potential to determine if specific sub-regions of the genome are being expressed. These can then be compared to the nucleic acid sequences present in a number of other human viruses.

4. VIRAL GENES AND GENE PRODUCTS IN HERPES SIMPLEX VIRUS-TRANSFORMED CELLS

Schaffer, P. A., Weller, S. K., Sidney Farber Cancer Center Inc., Dept. of Microbiology & Molecular Genetics, *44 Binney St., Boston, Massachusetts*, 02115, United States of America

The purpose of this investigation is to identify the HSV DNA sequences and gene products in HSV-transformed cells and to isolate and characterize transformation-defective mutants of the hr-t class in an effort to identify essential transforming genes of HSV. The following series of studies is proposed: A series of viral-antigen-positive cell lines transformed in vitro by HSV-1 and -2 antigen-positive cervical carcinoma cell lines will be characterized with regard to the viral DNA sequences which they contain in order to identify the set of DNA sequences common to all lines. Virus specific mRNA will be a) isolated from transformed cells by hybridization to labelled viral DNA fragments and b) translated in vitro in order to identify viral proteins encoded therein. Viral proteins will be identified in transformed cells by immunofluorescence and immunoprecipitation tests using potent antisera to HSV-infected and transformed cells, and to individual viral polypeptides. The map locations of DNA templates specifying proteins found in all or most transformed cell lines will be determined by hybrid-arrested translation of viral mRNA from transformed cells and by the analysis of HSV-1 x HSV-2 recombinants generated in crosses between pairs of HSV-1 and -2 ts mutants available in this laboratory. Intact viral DNA and DNA fragments corresponding to a) the DNA sequences in transformed cells and b) the sequences specifying potential 'transforming' proteins will be mutagenized in vitro and mutations will be rescued by coinfection of susceptible cells with intact wild-type virus DNA. Mutants able to replicate in HSV-transformed cells but not in

normal cells (hr or host-range mutants) will be sought. Such mutants may be defective in essential transforming genes and thus, will be tested for their transforming ability. Transformation-defective hr mutants (hr-t mutants) will then be characterized genetically and biochemically in order to elucidate the function of the mutant gene.

5. PRESENCE OF HERPES SIMPLEX VIRUS INFORMATION IN HSV TRANSFORMED CELLS AND IN BIOPSY SPECIMENS BY IN SITU HYBRIDIZATION

Brown, G., Biswal, N., Levy, C. C., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Molecular Biology Laboratory, *Baltimore, Maryland*, 21201, United States of America

Radiolabeled DNA and its restriction endonuclease fragments are being used in hybridizations to determine which regions of the HSV genome are present in HSV transformed cells and in cervical biopsy specimens from normal donors and those with dysplasia and carcinoma.

6. SITE SPECIFIC MUTAGENESIS IN THE GENE FOR THYMIDINE KINASE OF HSV

Summers, W. C., Yale University, School of Medicine, Dept. of Therapeutic Radiology, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

This is part of a broader project. A summary of this subproject is not available.

7. HERPES SIMPLEX VIRUS THYMIDINE KINASE GENE

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OBJECTIVES: Use clones, characterized cell lines biochemically transformed by HSV-2 and HSV-1 to form human-mouse somatic cell hybrids. Select for hybrid cells using suitable selective media. Analyze subclones of the somatic cell hybrids to verify the presence of herpes virus TK in the hybrid cells. Correlate the presence of individual human chromosomes in clonal sublines of hybrid cells with the presence of HSV-specified TK. Conduct biochemical tests for the presence of human isozymes to complement the karylogical identification of human chromosomes in hybrid subclones.

8. INTEGRATION, EXCISION AND REARRANGEMENT OF GENETIC INFORMATION

Enquist, L., Thompson, K. D., Brownell, G., Madden, M. J., Vande Woude, G. F., Weisberg, R., Sternberg, N., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Virus Tumor Biochemistry Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Our principal effort is directed toward understanding the mechanisms by which novel DNA rearrangements occur in vivo. We have concentrated on two major systems: bacterial viruses capable of integration and excision and herpes simplex virus (HSV). Phage lambda is used for both in vivo and in vitro recombination systems and for cloning DNA fragments of HSV. We are studying the integration of lambda into secondary sites on the E. coli genome. We can now screen lambda integration mutants for markedly altered integration patterns. We have isolated a lambda mutant that integrates at high frequenies in E. coli lacking the normal integration site. We are isolating specificity mutations in phage integration proteins, as well as deletions that define the control and structural regions of the lambda integration system. We are constructing plasmid chimeras in vitro that overproduce integration and excision proteins.

We are investigating other bacterial systems with temperate viruses to further our insight into DNA integration mechanisms. We have cloned many fragments of the HSV genome in lambda and plasmids and are analyzing the structure of specific HSV fragments.

BIBLIOGRAPHIC REFERENCES: Enquist, L.W. and Skalka, A.: Replication of bacteriophage lambda. Trends in Biochem. Sciences 3: 279-282, 1979. Enquist, L.W., Madden, M.J., Shiop-Stansly, P., and Vande Woude, G.F.: Cloning of herpes simplex type I DNA fragments in a bacteriophage lambda vector. Science 203: 541-544, 1979.

9. MECHANISMS OF VIRAL INFECTION IN RELATION TO CANCER

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This application is for continued support (years 16-20) of research on herpes simplex virus and on cells productively infected with this virus. The application lists 3 projects that concern the structural and functional organization of the viral genome.

The aim of the first project is to delineate the organization of viral genes in transcriptional units. We propose to delineate on the physical map of HSV DNA the 5', 3' and intervening sequences of viral mRNA made early in infection (alpha RNA) and of mRNA specifying structural polypeptides late in infection (gamma RNA).

The aim of the second project is to elucidate the function of a gene which appears to control the expression of all early functions. The project centers on the properties of a ts mutant which does not shut off the host and does not specify any proteins at the non-permissive temperatures.

The aim of the third project is to test specific aspects of the model for replication of HSV-DNA. The project entails transinfection of cells with DNA whose termini have been altered by addition of viral sequences and circularization.

BIBLIOGRAPHIC REFERENCES: Bartkoski, M.J., Jr. and Roizman, B.: Regulation of herpesvirus macromolecular synthesis. VII. Inhibition of internal methylation of mRNA late in infection. Virol. 85: 146-156, 1978. Buchman, T.G. and Roizman, B.: Anatomy of bovine mammillitis virus DNA. I. Restriction endonuclease maps of four populations of molecules that differ in the relative orientation of their long and short components. J. Virol. 25: 395-407, 1978.

10. GENETIC STUDY OF ANIMAL VIRUSES

Summers, W. C., DasGupta, U., Wagner, M., Yale University, School of Medicine, Dept. of Therapeutic Radiology, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

We will continue our studies aimed at construction of mammalian cells with nonsense suppressors. We will construct, by in vitro methods, mutations in the anticodon region of cloned serine-tRNA genes from human DNA. These in vitro-made suppressor genes will be co-transformed into mammalian cells and tested for function with our previously characterized set of nonsense mutants in the TK gene of herpes simplex virus.

11. VIRAL ONCOLOGY RESEARCH CENTER PROGRAM

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Project I concerns the functional organization of the herpes simplex and Epstein-Barr virus genomes. The objectives of this project are to map and define the viral genes with specific emphasis on genes responsible for transformation of cells and for the maintenance of the transformed state. Project II concerns the expression of HEL 12 retrovirus. The objectives of this project are to characterize the gene product specified by this virus in human tissues by molecular probes and by immunologic approaches.

12. MOLECULAR BASIS OF VIRAL ONCOGENESIS

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We propose to continue studying mechanisms by which oncogenic viruses induce transformation. These approaches include: identification of the herpes simplex virus genes responsible for induction and maintenance of transformation; studies of the mechanism of transformation of human lymphocytes by Epstein-Barr virus; investigation of the mechanisms which regulate transcription of the early region of SV40; and characterization of endogenous genetic information related to avian RNA tumor viruses in uninfected chicken cells.

BIBLIOGRAPHIC REFERENCES: Thorley-Lawson, David A., and Strominger, Jack L. Reversible Inhibition by Phosphonoacetic Acid of Human B Lymphocyte Transformation by Epstein-Barr Virus. Virology 86:107-108, 1978.

13. BIOCHEMICAL ASPECTS OF VIRAL CARCINOGENESIS

Kit, S., Dubbs, D. R., Trkula, D., Otsuka, H., Qavi, H., Baylor College of Medicine, School of Medicine, Dept. of Virology & Epidemiology, *1200 Moursund Ave., Houston, Texas*, 77025, United States of America

This research involves: (1) Studies on human and mouse cells biochemically transformed to the thymidine kinase (TK)-positive phenotype by herpesvirus TK genes; and (2) studies on the significance of the 'early region' of the SV40 genome for the maintenance of the transformed phenotype.

A clone-purified Pvu II fragment of HSV-1 DNA, which codes for TK, is being used to analyze the interaction of the TK gene with RNA polymerase and the regulatory alpha protein, VP175, which is coded by HSV-1 (KOS) gene B. Since the Pvu II fragment also codes for a herpesvirus-associated nuclear antigen (HANA), experiments are being carried out to learn whether HANA maps to the right of TK, is on the same DNA sequence as TK but is translated from a different mRNA from the mRNA that codes for TK, or whether HANA and TK are one and the same. The marmoset herpesvirus restriction nuclease fragment that codes for TK has been identified. It will be cloned in an E. coli plasmid and its position on the physical map of marmoset herpesvirus DNA will be ascertained. Recombinants containing herpesvirus TK and SV40 DNA sequences will be used to biochemically transform human cells. Conditions will be sought which would permit the recombinant DNA to replicate extrachromosomally. The possible methylation of SV40 and herpesvirus TK DNA sequences in biochemically transformed cells will be studied.

14. ANATOMY OF HERPES SIMPLEX VIRUS DNA

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This is part of a broader project. A summary of this subproject is not available.

15. GENE ORDER AND REGULATION OF GENE EXPRESSION

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This is part of a broader project. A summary of this subproject is not available.

16. MAPPING OF VIRAL FUNCTIONS BY ANALYSIS OF DEFECTIVE VIRUSES

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This is part of a broader project. A summary of this subproject is not available.

17. MAPPING OF VIRAL FUNCTIONS BY IN VITRO PROTEIN SYNTHESIS

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This is part of a broader project. A summary of this subproject is not available.

18. MAPPING OF VIRAL FUNCTIONS

Roizman, B., University of Chicago, Pritzker School of Medicine, Dept. of Medicine, *5801 S. Ellis Ave., Chicago, Illinois*, 60637, United States of America

This is part of a broader project. A summary of this subproject is not available.

2. VIRUS REPLICATION

19. THE ROLE OF HERPESVIRUS GLYCOPROTEINS IN CELLULAR INTERACTIONS AND VIRAL PENETRATION

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The specific objective of our proposal is to investigate the roles of herpes simplex virus (HSV) glycoproteins in mediating viral entry of a host cell and in promoting cell fusion and dissemination of virus. Members of the herpesvirus group cause leukemias, lymphomas and carcinomas in animals and possibly also in man. Because the development of disease requires entry of the virus into susceptible host cells and the dissemination of infection to tissues in which malignant transformation occurs, an understanding of these events will be important in disease prevention.

At least four HSV-specific glycoproteins have been identified in the virion and in membranes of cells infected with HSV-1. Glycoprotein B2, present in detergent-stable heat-labile dimeric form, is required for virion infectivity and for the promotion of fusion between the plasma membranes of infected cells. Glycoprotein C2 appears to inhibit the fusion-promoting activity of B2, which explains why wild-type isolates of HSV usually do not cause extensive cell fusion. Our working hypothesis is (1) that membrane fusion is required for viral entry of a host cell as well as for cell fusion, (2) that glycoprotein B2 plays an essential role in the process of HSV-induced membrane fusion, both between the virion envelope and cell surface and between two cell surfaces, and (3) that C2 may be able to inhibit the initial stages of viral infection, as well as cell fusion, provided that C2 is present in the surface of a cell to which a virion attaches.

Based on our current knowledge and the systems available, the experiments proposed here will focus on three specific areas: (1) Antisera directed against individual glycoproteins will be tested for their effects on cell fusion and virion infectivity. (2) Experiments will be carried out to determine whether the function of glycoprotein B2 in infectivity and in cell fusion requires its dimeric conformation, by correlating heat dissociation of the dimer with possible loss of biological activity. (3) The topological requirements for C2 to suppress cell fusion will be studied. The effect of C2 expression by the host cell on viral penetration will be determined.

20. VIRAL RNA SYNTHESIS CONTROL IN HERPES VIRUS INFECTION

Wagner, E. K., Gaylord, B., Devi, G. B., Holland, L. E., Anderson, K. P., Tribble, L. L., Costa, R. E., University of California, Irvine Campus, School of Biological Sciences, Dept. of Biochemistry & Molecular Biology, *Irvine, California*, 92664, United States of America

The control of synthesis of herpes simplex virus (HSV) specific mRNA is being studied. We have developed techniques for the isolation, mapping, and in vitro translation of HSV-1 mRNA species. This supplemental request is aimed at maximizing our progress. Specific areas to be studied are: (1) The localization and direction of transcription of HSV-1 mRNA species; (2) detailed analysis of the structure of specific HSV-1 mRNA species; (3) study of the nuclear precursors of HSV-1 mRNA; (4) examination of the biological activity of HSV-1 transcripts.

Methods to be used include preparative hybridization of HSV-1 mRNA using viral DNA and restriction fragment bound to cellulose; study of possible interruption of mRNA sequences using S1 nuclease digestion; use of reverse transcriptase to make 3' cDNA probes to HSV-1 mRNA; electron microscopy of DNA-RNA hybrids; and various assays of biological activity.

BIBLIOGRAPHIC REFERENCES: Holland, L., Anderson, K., Shipman, C., and Wagner, E. (1980) Viral DNA synthesis is required for the efficient expression of specific herpes simplex virus type 1 mRNA species. Virol., in press. Anderson, K., Holland, L., Gaylord, B., and Wagner, E. (1980). Isolation and translation of mRNA encoded by a specific region of the HSV-1 genome. J. Virol. in press.

21. STUDIES OF THE BIOGENESIS OF THE HERPES SIMPLEX VIRUS MRNA ENCODING THYMIDINE KINASE

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OBJECTIVES: The biogenesis of mRNA will be studied using a model system which allows combined biochemical and genetic analysis: the herpes simplex virus (HSV) gene for thymidine kinase (TK). TK is a dispensable function, and a large number of viable TK negative mutants of HSV have been isolated. Both the TK positive and TK negative phenotypes can

be made conditionally lethal, allowing detailed genetic studies. This ability, coupled with the availability of a small fragment of HSV DNA containing the TK gene, will allow a dissection of the structure of the TK gene, and of the processes by which mature TK mRNA is generated from its nuclear precursor. The objectives of this study are two-fold: a. To characterize the normal mechanism of TK mRNA synthesis, and b. To determine which of the steps leading to the formation of TK mRNA are subject to inactivation by mutation.

APPROACH: The DNA sequences encoding mature TK mRNA will be precisely mapped on the HSV1 genome, using physical techniques which are designed to disclose the positions of any 'splice' points on the RNA. Nuclear RNAs bearing TK negative related sequences will be similarly characterized, and the precursor-product relationships between these RNAs, and between them and mature TK mRNA will be studied.

A series of TK negative mutants (including deletion mutants) which fail to synthesize an intact polypeptide will be screened to identify those which fail to accumulate intact TK mRNA. Such mutants may be defective in some stage of intranuclear RNA processing, or blocked at the level of transcription. These possibilities will be distinguished by characterizing the TK negative related RNAs induced by the mutants. A comparison of these RNAs with those induced by wild-type virus may indicate the nature of the defects, and should provide information about the wild-type pathway for mRNA synthesis. Mutations with interesting effects on TK mRNA synthesis will be ordered with respect to other mapped TK negative mutants by standard genetic crosses. The map positions of such mutants will identify some of the DNA sequences which control the transcription and processing of TK mRNA.

22. METABOLISM OF CELLS INFECTED WITH NUCLEAR DNA VIRUSES

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This proposal is concerned with the following problems: (1) The elucidation of the transcriptional controls operating in various cell lines infected with different types and strains of herpes simplex virus (HSV). (2) The identification of the functions of the 'immediate-early' proteins of the herpes viruses and the exploration of the use of antisera against these proteins as probes to detect viral products in cells experimentally transformed by HSV and pseudorabies (Pr) virus and in cells putatively naturally transformed by HSV. (3) The characterization of interactions of HSV, types 1 and 2, and of Pr virus with cells transformed by RNA tumor viruses. (4) The characterization of the viral glycoproteins excreted by infected cells. Special emphasis on the possible use of these glycoproteins as diagnostic tools. (5) The characterization of the DNA molecules present: (a) In populations of recently plaque-purified virions, (b) in standard virions passaged several times at low multiplicity, (c) in several different isolates of populations of defective, interfering virions. (6) The characterization of the replicative forms of the viral DNA in cells infected with the virions listed in (5).

BIBLIOGRAPHIC REFERENCES: Feldman, L., Rixon, F.J., Jean, J.-H., Ben-Porat, T. and Kaplan, A.S. (1979). Transcription of the genome of pseudorabies virus (a herpesvirus) is strictly controlled. Virology 97, 316-327. DeMarchi, J.M., Ben-Porat, T. and Kaplan, A.S. (1979). Expression of the genome of noninfectious particles in stocks of standard and defective interfering pseudorabies viruses. Virology 97, 457-463.

23. HERPES SIMPLEX VIRUS GENE REGULATION

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The objective of this work is to characterize the process of viral mRNA biogenesis and its control in cells infected with herpes simplex virus 1 (HSV-1). Specifically this includes characterization of 1) the size, genome locations and organization of viral transcription units, 2) the size, structure, polypeptide coding, and genome mapping of the viral mRNAs, and 3) the processing of the primary viral transcripts.

The goals for the coming year are: a) Characterization of delayed-early (DE) mRNAs. By using drugs and temperature sensitive mutants to restrict transcription to early genes, we will identify and separate DE mRNAs by hybridization to HSV-DNA cellulose and by electrophoresis in gradient, denaturing gels. The isolated RNAs will be characterized by in vitro translation and by Southern blot and R-loop mapping techniques. b) UV mapping of early viral mRNAs. The sensitivity of synthesis of individual mRNAs to UV irradiation of the infecting virus or the infected cell will be studied. This will provide data on the early viral transcription units and information on possible co-transcriptions of viral genes. c) Processing of viral primary transcripts will be investigated by two approaches. The first will consist of selective enzymatic digestion of hybrids formed between unlabeled mRNAs and labeled restriction fragments of viral DNA followed by electrophoretic analysis of the products in neutral and alkaline gels (Berk and Sharp technique). The second will be 'R-loop' mapping of mRNA and HSV-1 DNA restriction fragments by electron microscopy.

24. BIOCHEMISTRY OF HERPESVIRUSES

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This application is for continued support of studies on biochemistry of herpes simplex viruses particularly as it relates to cancer with respect to the following: (1) Detailed analysis of the factors determining the transcriptional and post-transcriptional regulation of viral RNA function in productive infection described by us earlier, (2) continuation of the current studies on the translational program of the virus and the characterization of the structural and non-structural proteins, (3) confirmation and expansion of our analyses of human cancers for viral DNA and RNA sequences and analysis of these sequences with respect to corresponding sequences made in productively infected cells, and (4) continuation and expansion of our analyses of cells transformed by herpes simplex type 1 and 2.

25. DNA SYNTHESIS IN HERPES SIMPLEX VIRUS

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This is part of a broader project. A summary of this subproject is not available.

26. STIMULATION OF DNA SYNTHESIS IN CELLS INFECTED BY HERPES VIRUSES

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OBJECTIVES: 1) To characterize conditions under which various herpes viruses, including herpes simplex virus, Marek's disease virus and herpes virus of turkeys, may reproducibly stimulate cellular DNA synthesis. 2) To investigate whether such DNA synthesis is associated with increased cell proliferation or with other processes such as repair. 3) To investigate whether such synthesis is stimulated by extracellular growth factors or by intracellular events following virus infection.

APPROACH: All experiments are carried out with cells in culture: 1) Cells that are known or suspected to be non-permissive for herpes virus replication, including glial cells, differentiated muscle and red blood cells, are infected with herpes virus and the rate of DNA synthesis determined following incorporation of 3H-thymidine. The type of DNA syntheis occurring is determined by separation of viral and cellular DNA on CsCl gradients. Similar experiments are carried out by incubating infected cells at temperatures that are non-permissive for virus replication but permissive for cell DNA synthesis. 2) In all experiments cell numbers are determined and DNA synthesis followed for several days following infection. Stimulation of DNA synthesis is studied in relation to the growth state of cells, i.e., in dense and sparse cultures and under conditions of serum starvation. 3) Used culture fluids from experimentally infected cultures are tested for their ability to stimulate growth of cells that have been deprived of serum. Attempts are being made to characterize growth stimulating material found in cultures infected by Marek's disease virus. The possibility that prostaglandins are involved in these processes is being studied by the addition of prostaglandins or inhibitors of prostaglandin metabolism to cultures under investigation.

27. POLYPEPTIDES INVOLVED IN HERPES SIMPLEX VIRUS REPLICATION AND CELL TRANSFORMATION

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Herpes simplex virus (HSV) induced proteins are being studied to determine their roles in virus replication and cell transformation. Sufficient characterization has been made to permit a tentative listing of 20 corresponding polypeptides of HSV-1 and HSV-2. Characteristics of two polypeptides associated with viral transformation have been described.

28. BIOCHEMISTRY OF HERPESVIRUS MEMBRANE PROTEINS

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The purposes of the studies proposed here are to characterize the membrane glycoproteins specified by herpes simplex virus (HSV) and, in particular, to define their roles in viral entry of a host cell and in HSV-induced cell fusion, two processes that are probably governed by overlapping sets of viral gene products. In addition, we intend to investigate the role in viral replication of the HSV-induced Fc-binding activity, which we have shown to be associated with a specific glycoprotein. Some of the approaches to be used include the preparation of purified solubilized glycoproteins for investigation of their individual activities; isolation and characterization of mutants with specific alterations in HSV glycoproteins and/or virion infectivity; characterization and genetic analyses of viral mutants that express the capacity to induce cell fusion as a result of the mutation; characterization of the interaction between immunoglobulin and the HSV-induced Fc-binding glycoprotein and assessment of its capacity to bind to other molecules of interest.

29. HERPESVIRUS PROTEIN SYNTHESIS AND VIRION ASSEMBLY

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Herpesviruses are widespread in nature and associated with both latent and neoplastic infections. At least five, including herpes simplex viruses 1 and 2, herpes zoster virus, cytomegalovirus, and Epstein-Barr virus, infect man and three of these are implicated as etiologic agents in human neoplasias. The objective of this research is to examine in greater detail the flow of events involved in the assembly and maturation of herpes simplex virus, and to identify specific gene functions that regulate this process. Intermediates in virus assembly will be isolated and their composition and topological organization analyzed by sensitive techniques of two-dimensional polyacrylamide gel electrophoresis, two-dimensional peptide analysis, and in vitro surface radiolabeling. In addition, efforts will be made to establish the role of modifications such as protein phosphorylation and cleavage in the assembly process. The general rationale of these experiments is that knowledge of what occurs during productive infection may lead to a better understanding of what transpires in the course of abortive infection, from which virus-transformed cells might ultimately be derived.

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30. BIOCHEMICAL EVENTS IN TUMOR VIRUS REPLICATION AND TRANSFORMATION

Vande Woude, G. F., Robey, W. G., Ascione, R. A., Enquist, L. W., McClements, W. L., Thompson, K. D., Fischinger, P. J., Blair, D. G., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Virus Tumor Biochemistry Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

Internal endonuclease restriction fragments of herpes simplex virus type 1 (HSV-1) representing 70% of the viral genome have been cloned in bacteriophage lambda (AR no. Z01-CP-04914-02-LMV). These include even the large Eco R1 D,G,H and I fragments up to 17,000 b.p. in size. Each cloned fragment has been characterized by Southern transfer hybridization. More than 40 clones have been obtained of the heterogeneous defective DNA (dDNA) of HSV-1 WES-gt recombinant hybrids (AR no. Z01-CP-05102-01-LMV). From endonuclease restriction digests, three major classes of dDNA have been identified. The heterogeneity in each of these classes appears to result from a novel joint formed between the rightmost end of HSV-1 and a variable position within the S unique region of the HSV-1 parent.

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31. LATENCY IN HERPES VIRUS INFECTION OF CELLS AND TISSUES

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Acute and recurrent diseases caused by herpesviruses are among the most common and often most serious infections in man. Herpes simplex virus and cytomegalovirus are two of the most important of these agents. We propose to further define the pathogenesis of these viruses, particularly the mechanisms associated with latent and recurrent infections, by employing herpes simplex virus and murine cytomegalovirus in the mouse and in cell culture systems.

In the herpes simplex studies, temperature sensitive mutants of the virus with defined phenotypes will be employed in mice to determine biochemical events necessary for establishment of latent infections. In addition, extensive attempts will be made to establish latent infections in cultures of differentiated neuroblastoma cells with both wild-type and temperature sensitive viral mutants.

We have established latent cytomegalovirus infections in mice, and have succeeded in reactivating these infections with antilymphocyte serum and cortisone. Now, we propose to extend these findings by 1) defining the cell-types involved in harboring latent virus, 2) determining the conditions and mechanisms by which reactivation occurs, and 3) investigating the efficacy of various therapeutic regimens.

BIBLIOGRAPHIC REFERENCES: Bak, I.J., Markham, C.H., Cook, M.L., and Stevens, J.G. Ultrastructural and Immunoperoxidase Study of Striatonigral Neurons by Means of Retrograde Axonal Transport of Herpes Simplex Virus. Brain Research: 143: 361-368 (1978).

3. CELLULAR TRANSFORMATION

32. ONCOGENIC TRANSFORMATION STUDIES OF HSV-1 AND HSV-2

Macnab, J. C., University of Glasgow, Inst. of Virology, *Church St., Glasgow G11 5JR, Scotland, United Kingdom*

OBJECTIVE: The object of this study is to confirm the oncogenic potential of HSV in transformation and tumor studies and determine which genome fragments are responsible for expression of information and also for initiation and maintenance of the transformed state.

APPROACH: A system has been established to monitor in vitro transformation and oncogenicity in rats and hamsters. To date, expression has been monitored by immunofluorescence studies, complementation with superinfecting ts mutants and recombination with superinfecting ts mutants.

PROGRESS: Progress has been accomplished as explained in the approach. At present, studies to establish those antigens expressed in transformation, the fragments initiating and maintaining transformation and the total genome complement which may be present are in progress.

33. STUDIES OF CELLULAR TRANSFORMATION BY HSV-2

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OBJECTIVES: Identification of the minimal portion of HSV-2(333) DNA that is necessary and sufficient to produce morphological cell transformation in primary rat embryo cells and Swiss 3T3 cells. Extension of this work to human cells and use of several different strains of HSV-1 and HSV-2 DNAs. Establishment and passaging of transformed cell lines containing different segments of HSV DNA for the purpose of studying the role, if any, of the viral specified products in maintaining the transformed phenotype. Preparation of high specific activity radiolabeled probes of the herpesvirus 'transforming gene' DNA, preferably in the form of seperated single strands suitable for use in high sensitivity screening of transformed cell lines and tumor tissues for the presence of viral DNA or RNA. Use of the high sensitivity probe DNA to test for possible specific sites of integration of the viral DNA into the host cell genome either within 'fragment'-transformed lines or within those already available that were produced using whole inactivated virus.

34. HERPES VIRUS EXPRESSION IN TRANSFORMATION AND LATENCY

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The overall objective of this project is to determine how herpes simplex virus (HSV) persists in cells which are either latently infected or which have become morphologically transformed as a consequence of an interaction. Specifically we wish to define which sequences of HSV-2 DNA code for products which are sufficient to alter the phenotype of cells; determine the arrangement of the viral sequences which are retained; and analyze the viral gene products. Additionally, we would like to determine whether expression of particular viral functions is necessary to maintain the HSV genome in a latent state in neurons.

BIBLIOGRAPHIC REFERENCES: Galloway, D.A., Copple, C.D., McDougall, J.K. (1980). Analysis of viral DNA sequences in hamster cells transformed by herpes simplex virus type 2. Proc. Nat. Acad. Sci. 77:880-884. McDougall, J.K., Galloway, D.A., Fenoglio, C.M. (1980). Cervical carcinoma: detection of herpes simplex RNA in cells undergoing neoplastic change. Int. J. Cancer 25:1-8.

35. TRANSFORMING GENES OF HERPES SIMPLEX VIRUS

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The objective of the proposed research is to identify the viral genes involved in herpes simplex virus (HSV)-induced transformation and to elucidate their role in the transformation process. For this purpose, the HSV genome will be cleaved using restriction endonucleases and transformation will be attempted with specific HSV DNA fragments. This method has been used to demonstrate both biochemical and morphologic transformation by individual viral DNA fragments. The identity of essential viral replicative genes encoded by known and newly identified transforming fragments will be determined using ts mutants in marker rescue experiments. Additional genes encoded by fragments will be identified by mutagenesis of the fragment itself and by rescuing induced mutations through coinfection with wild-type virus DNA. Both temperature-sensitive (ts) and specialized mutants, defective in the expression of individual viral antigens, will be isolated. Whether each gene encoded by the transforming fragment is essential for transformation will be determined by attempting to induce transformation using HSV DNA fragments bearing ts or specialized mutations. The functions of essential transforming genes will be elucidated by phenotypic characterization of a) the ts and specialized mutants whose functions are essential for transformation and b) the cells transformed by fragments bearing these mutants. The ability of viral gene products not essential for transformation but expressed in HSV-transformed cells, to alter the phenotype of HSV-transformed cells, will also be examined.

36. PROTEINS OF HSV-INFECTED AND TRANSFORMED CELLS

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The objectives of the proposed research are to prepare highly purified and specific immunological probes to be used in further defining virus gene expression within herpes simplex virus type 1 (HSV-1)- and type 2 (HSV-2)-infected and transformed cells. The nonglycosylated proteins obtained from purified virions and from infected cells will be fractionated and purified by two general approaches. First, HSV-specific polypeptides will be purified by high resolution preparative polyacrylamide gel electrophoresis containing sodium dodecyl sulfate. These highly purified polypeptides will be further purified and analyzed by two-dimensional gel electrophoresis. Second, HSV-specific native proteins will be purified utilizing several techniques which include hydroxylapatite chromatography, isoelectric focusing and affinity chromatography. Monospecific antisera to the purified polypeptides and native proteins will be produced in rabbits. These antisera will be used in studies to further characterize the proteins synthesized in the infected cell incorporating the use of immunofluorescence, immunoprecipitation, radioimmunoassay, and immunoperoxidase staining. In addition, these sera will be used extensively to define the expression of HSV specific genes within the transformed cell. It is postulated that such highly specific immunological probes will eventually be of significant value in further defining the role certain HSV proteins may play in the latently infected cell as well as in the cancer cell.

37. GENETICS OF HERPES VIRUS TRANSFORMATION

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The objective of this proposed research is to determine the identity, structure and expression of the herpes simplex viral genes involved in the alteration of cellular growth properties by transformation of the cells. The region of the herpes simplex virus type 1 (HSV-1) genome capable of transformation has been identified, but no viral mutants have been isolated in this viral function. For this reason we propose a detailed genetic study of the transformation region by the directed mutagenesis of this region. We will isolate temperature-sensitive mutants and host-range mutants in this region of the genome. We also plan to isolate new early mutants that regulate the expression of the transforming gene(s).

We specifically propose to 1) mutagenize in vitro restriction endonuclease DNA fragments from the transforming region of the genome and re-introduce the DNA fragments into an infectious genome by marker rescue; 2) screen for and characterize temperature-sensitive mutants and host-range mutants; 3) insert DNA sequences from the transforming and early gene regions into new regions of the genome in an attempt to determine how the expression of these gene sequences is regulated.

The goal of these studies is to identify each of the viral genes involved in cellular transformation and to assign the precise molecular function to each. The correlation of a defect in the biochemical properties of the proteins encoded by these genes with the lesions introduced will allow the assignment of these functions.

38. HERPESVIRUS GENE EXPRESSION IN TRANSFORMED CELLS

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The long-range objective of this project is to define the factors that govern the expression of viral genes in cells transformed by herpes simplex virus (HSV). Attention will be focused initially on regulation of the HSV genes for thymidine kinase and a membrane glycoprotein, because of the availability of transformed cell line derivatives that differ in the levels of expression of these genes. One of the aims of this proposal is to determine whether changes in the expression of a viral gene in

transformed cells correlate with deletions or rearrangements of the viral DNA sequences. A second aim is to characterize structural features of mRNAs produced from selected regions of the viral DNA sequences, in comparison with the mRNAs made during productive infection, in order to identify structural differences that reflect differences in the transcriptional and post-transcriptional processes required for production of a functional viral gene product in transformed and infected cells. A third aim is to investigate further the basis for enhanced levels of resident viral gene expression in transformed cells super-infected with HSV and also for a recent finding that synthesis of some cellular proteins in transformed cells is enhanced by HSV super-infection, in contrast to the inhibition of cellular protein synthesis that usually accompanies HSV infection. Methods to be used include digestion of transformed cell DNA with restriction endonucleases, fractionation by electrophoresis and transfer to nitrocellulose filters for identification by hybridization of fragments containing viral DNA sequences. Viral mRNAs will be enriched by hybridization to viral DNA fragments on cellulose, fractionated by electrophoresis by estimations of size, and individual species recovered to identify precisely the viral DNA sequences to which each is homologous and to test messenger activity in vitro. The expression of specific viral and cellular proteins after super-infection of transformed cells will be monitored by enzyme assays and by immunoprecipitation with antisera produced in syngeneic hamsters against HSV-transformed hamster cells.

BIBLIOGRAPHIC REFERENCES: Spear, P.G. 1979. Herpesvirion envelopes and infected cell membranes. In: Cell Membranes and Viral Envelopes, edited by H.A. Blough and J.M. Tiffany. Academic Press, New York, in press. Sarmiento, M., Haffey, M. and Spear, P.G. 1979. Membrane proteins specified by herpes simplex viruses. III. Role of glycoprotein VP7(B2) in virion infectivity. J. Virol., in press.

39. BIOCHEMICAL EVENTS IN TUMOR VIRUS REPLICATION AND TRANSFORMATION

Vande Woude, G. F., Rabey, W. G., Ascione, R. A., Enquist, L. W., McClements, W. L., Thompson, K. D., Fischinger, P. J., Blair, D. G., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Virus Tumor Biochemistry Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The summary for this project is under entry no. 30.

40. POLYPEPTIDES INVOLVED IN HERPES SIMPLEX VIRUS REPLICATION AND CELL TRANSFORMATION

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The summary for this project is under entry no. 27.

41. RELATION BETWEEN HERPES SIMPLEX VIRUS TYPE 2 INFECTION AND HUMAN CERVICAL CANCER

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OBJECTIVE: The main goal of this research project is to identify herpes simplex virus type 2 DNA sequences involved in oncogenic cell transformation and to study their persistence in human cervical carcinoma cells. Additionally, virus specific products of the 'oncogene' region will be identified to investigate their persistence in tumor cells and their immunological response in sera of cervical carcinoma patients during the course of the disease.

APPROACH: Different cell systems will be infected with HSV-2 DNA by using the calcium method to obtain cell lines that are biochemically and/or morphologically transformed. By employing preparative agarose gel electrophoresis, viral DNA fragments will be prepared. Purified fragments will be characterized with respect to their biological and biochemical properties. With the aid of the Southern blotting technique and in situ hybridization, herpes specific DNA and RNA sequences will be detected in morphologically transformed cells and human cervical carcinoma cells. By using a combination of electrophoresis and immunology, detection of viral specific products will be carried out in these cells. Sera from cervical carcinoma patients will be assayed for antibodies against virus specific products originating from the 'oncogene' region. During the course of the disease the levels of these antibodies will be followed.

PROGRESS: Initially, sera of patients with cervical cancer and healthy matched controls were investigated on the presence of HSV-2 neutralizing antibodies by determination of the Index II/I. It also appears that in The Netherlands antibodies against HSV-2 are more frequently present in patients with cervical cancer than in controls. This Index II/I was again determined three years after treatment and revealed that there was no simple correlation between increase of HSV-2 neutralization titres and the course of the disease. A new method for isolation of large amounts of HSV-2 DNA from total lysate of infected cells was developed. This DNA was further characterized by electron microscopy, fragmentation with bacterial restriction enzymes and biochemical transformation capacity and appeared useful for the further analysis of the genetic information of the HSV-genome (Walboomers, J. and Ter Schegget, J., Virology 74, 256-258, 1976). (Text Abridged.)

42. A PROGRAM IN VIRAL ONCOLOGY

Rawls, W. E., Bacchetti, S., Branton, P., Graham, F., Smiley, J., McMaster University, *1200 Main St. W., Hamilton, Ontario*, L8S 4L8, Canada

OBJECTIVE: To determine the mechanism by which viruses induce oncogenic transformation and to examine the role of viruses in human cancers.

APPROACH: Studies of the molecular basis of viral transformation are being undertaken using adenovirus type 5 (Ad5), Rous sarcoma virus (RSV), and herpes simplex virus type 2 (HSV-2). The hypothesis being examined is that viral genetic information is inserted into host cell DNA and the gene products coded for by the integrated viral nucleic acid account for the transformed phenotype. This information will serve as a basis for evaluating the role of viruses in human carcinogenesis.

PROGRESS: The transforming ability of Ad5 has been found to involve the integration and expression of the 14% of the left end of the virus DNA. Two gene functions have been delineated in this region of the genome using host range mutants. Available evidence suggests that one gene (group I mutants) may regulate the production of a protein (58K) which is coded for by a second gene (group II mutants).

RSV has been found to alter the levels of cAMP-dependent protein kinase activity in transformed cells. In addition, differences exist in the phosphorylation of proteins of transformed cells as compared to normal cells. These observations complement the findings of others that the transforming gene product of RSV is a protein kinase. We have generated data suggesting that the transforming gene product of Ad5 also has protein kinase activity.

We have examined the putative role of HSV-2 in cervical cancer using a seroepidemiological approach and conclude that the virus may cause a portion of the cancer cases; this proportion varies from population to population. Initial attempts to evaluate the mechanism of transformation of the HSV-2 at the cellular level suggest, in confirmation of others, that stable integration of the viral DNA may not occur. However, our studies involved biochemical transformation with the thymidine kinase gene and this may not be comparable to oncogenic transformation.

43. REGULATION OF GENE EXPRESSION IN HERPES VIRUS

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This project aims at examining the possible role of herpes simplex virus alpha polypeptide ICP4 in modulating the expression of transforming gene in transformed cells.

My approach is to clone the ICP4 gene into bacterial plasmid DNA and generate site-specific mutants in vitro. The wild-type and mutant DNA will then be used together with TK DNA in co-transformation studies. The possible effect of ICP4 on the transformation efficiency and expression of transforming TK gene will be determined.

Possible enzymatic activity for ICP4 will be sought as it will provide a molecular basis for its controlling function.

44. MONITORING OF HERPES SIMPLEX VIRUS TUMOR-ASSOCIATED ANTIGENS IN HUMAN MALIGANT NEOPLASIAS

Tarro, G., DiGioia, M., Battista, A., Smeraglia, R., Cocchiara, R., Flaminio, G., Papa, C., Universita degli Studi di Napoli, Cotugno Hospital, Dept. of Virology, *Quagliariello, Naples, Italy*

The detection of HSV nonvirion antigens in selected tumors as expression of a repressed viral genome proves a continuing release of virus specific messages and supports an important role of the virus in the development of the tumor.

The purification of nonvirion antigen induced by HSV facilitates the detection of antibody for HSV nonvirion antigens in patients with certain cancers without absorption of their sera with HSV virion antigens. Furthermore, the preparation of specific antibody for these special antigens (nonvirion) is now relatively at hand in many laboratories for the investigation of the immune response of cancer and control populations.

PURPOSES AND AIMS: 1. To obtain purified preparations of HSV tumor-associated ('nonvirion') antigens (TAA) for use in radioimmunoassay and ELISA with cancer and control sera. 2. To monitor the presence of specific TAA antibodies in patients with different tumors of the oral and urogenital tract. 3. To correlate the clinical stage of the neoplastic disease with the presence of antibody to HSV-TAA. 4. To determine the difference in the amounts of HSV-TAA antibody between pre-operative patients and those clinically cured.

45. ISOLATION AND CHARACTERIZATION OF HOST-RANGE MUTANTS OF HERPES SIMPLEX VIRUS TYPE II AND ADENOVIRUS

Frost, E. H., Institut du Cancer de Montreal, *1560 Est Rue Sherbrooke, Montreal, Quebec*, H2L 4M1, Canada

A special class of mutants of adenovirus (Ad) and polyoma (Py) have been selected for growth in cells transformed by these viruses but not in untransformed cells. These mutants, called hr mutants, are situated in the transformation genes and have lost the ability to cause stable transformation. Due to its relevance to human cervical cancer I would like to isolate similar mutants from HSV-2 and characterize them in the following manner: 1) Locate the mutation: This will provide evidence for the location of the viral transformation sequences. I intend to confirm this by transforming cells with separated restriction endonuclease fragments of DNA. This information will be valuable in directing the search for viral markers (DNA, RNA, proteins) in transformed and cancer cells. 2) Viral growth properties: In order to understand the role of the viral transformation gene in lytic infection I will determine the hr mutant's ability to synthesize viral DNA and, in collaboration with Dr. Martha Suh, viral polypeptides. I will also determine the ability of the mutants to transform cells using DNA fragments to prove that the mutation is in the transformation sequences and not in a regulatory gene. 3) Host range of the mutants: Ad and Py hr mutants grow in some transformed cells but not in others indicating either that the transformation character necessary for hr mutant growth is not expressed in all lines and/or some cell lines are partially resistant to viral growth. These alternatives can be studied in the Ad system where transformation character negative (HeLa) and resistant (Ad transformed 14b) cells exist. I propose to locate the block in resistant cells to distinguish them from those not expressing the necessary transformation character. I will then attempt to overcome this resistance. This knowledge will then be applied to the study of the host range of HSV-2 mutants, particularly their interaction with cervical carcinoma cells which may be transformed and/or rendered resistant by HSV-2. The examination of the growth potential of HSV-2 hr mutants in a number of cell lines, including organ cultures, is proposed to point out specificity or reveal a common transformation character.

46. HERPESVIRUS CELL SURFACE ANTIGENS AND TUMORIGENICITY

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This project involves a genetic and immunological study of the interactions of herpes simplex virus type 1 (HSV-1) cell-surface antigens induced in infected and transformed hamster embryo fibroblasts with the immune system. Special attention is paid to (1) the role of these antigens in complement-dependent and cell-mediated immune cytolysis and (2) the role of virus-induced Fc receptors in blocking immune cytolysis. Evidence has been provided that virus-induced Fc receptors on infected and transformed cells can combine with Fc fragments, agg-IgG and immune complexes to block cell-mediated and complement-dependent immune cytolysis. Fc receptors in vivo may combine with blocking antibody or immune complexes, thus protecting tumor cells against the host's immune system. Genetic, biochemical and immunological techniques are employed to isolate, identify and characterize the virus-specified antigens. Hamster embryo cells infected with wild-type HSV-1 (KOS) and its temperature-sensitive mutants are analyzed for their spectrum of cell-surface antigens. Some mutant-infected cells vary in their surface antigen content, including Fc receptors, under nonpermissive conditions. Surface antigens radioiodinated by the lactoperoxidase technique are isolated by tandem analytical polyacrylamide gel electrophoresis and isoelectric focusing. In situ immune complexing procedures are performed in polyacrylamide gels using 125I-labeled specific antibody and Fc fragments to identify HSV antigens and Fc receptors.

47. HERPESVIRUSES - IMMUNOLOGIC AND BIOLOGIC STUDIES IN RELATION TO HUMAN TUMORS

Hampar, B., Vande Woude, G., Khoury, G., Nonoyama, M., Derge, J. G., Boyd, A. L., Zweig, M., Rabin, H., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Microbiology Section, *Frederick, Maryland*, 21701, United States of America

The long-range purpose of the project is to study the relationship between herpesvirus and human cancer. The major topic of interest concerns the mechanism of in vitro cell transformation by herpes simplex virus (HSV) and the putative cocarcinogenic role of HSV reflected in its ability to alter expression of endogenous type C virus information.

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48. MOLECULAR BIOLOGY OF HERPES VIRUS

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The objective of this proposed research is to define, in molecular terms, the mechanisms that control the expression of herpes simplex virus (HSV) genes in transformed cells. The proposed studies center on the expression of HSV thymidine kinase (TK) in biochemically transformed cells. In particular, we are interested in studying the relationship between information content and expression. This will be done by making available to mouse LTK negative cells various length restriction endonuclease generated fragments of HSV-1 DNA that contain the TK gene. Cells that have stably acquired the TK positive phenotype will then be examined for viral DNA sequences, and transcripts from the now resident viral DNA.

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49. REGULATION OF DIFFERENTIATION IN HEPATOCYTES IN VITRO

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The objective is to study the interaction of collagenase-isolated adult rat hepatocytes with viruses in order to achieve the two separate but related goals of determining the effect of virus infection and transformation on expression of differentiated liver cell functions, and the influence of a differentiated nonproliferating adult hepatocyte on virus replication. These goals will be achieved using rat hepatocytes transformed with wild type and temperature sensitive mutants of SV40 and herpes simplex virus type 2 (HSV-2)-infected adult hepatocytes, respectively. We have generated SV40 transformed hepatocytes, all of which were 100 percent positive for expression of SV40 tumor (T) antigen, but were diverse in expression of specific liver proteins. We propose to use temperature shift to analyze hepatocytes transformed by an SV40 temperature sensitive mutant for loss and acquisition of liver cell proteins and enzymes in parallel with altered characteristics of transformation and expression of T antigen. We will also study the effects of virus infection as well as transformation on expression of differentiated liver cell functions; in particular, expression of albumin and alpha-fetoprotein. We have also established that HSV-2 infection of adult rat hepatocytes is semipermissive and provides a unique system in which virus DNA was synthesized, virus particles were found in the nucleus, host protein synthesis was decreased, but hepatocyte DNA synthesis was stimulated. We propose to analyze the mechanism by which HSV-2 fails to permissively infect but does stimulate host DNA synthesis in an adult rat hepatocyte.

SV40 transformed hepatocytes provide continuous lines of cells expressing liver functions which can be used for gathering insight into eukaryotic cell control. Hepatocytes transformed by a temperature sensitive SV40 mutant may provide us with a mechanism by which differentiation can be reversed simply by altering temperature. The HSV-2 infected adult hepatocyte provides a system in which the effect of a differentiated cell type on replication of a virus which can establish both transformation and latent infections can be analyzed.

50. DEFECTIVE HERPES SIMPLEX VIRUS DNA

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This is part of a broader project. A summary of this subproject is not available.

B. EPSTEIN-BARR VIRUS

1. STRUCTURE AND GENOMIC ORGANIZATION

51. STRUCTURE OF THE EPSTEIN-BARR VIRUS GENOME

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OBJECTIVE: Characterization of intracellular EB virus DNA in human lymphoid cell lines, with regard to the replication mechanism of episomal circular viral genomes, DNA sequence differences between different EBV isolates, and integration sites.

APPROACH: The episomal viral DNA, or integrated viral DNA sequences, are purified and characterized by standard methods of molecular genetics (nucleic acid hybridization, restriction enzyme cleavage, gel electrophoresis, etc.).

52. STRUCTURE AND EXPRESSION OF THE EPSTEIN-BARR VIRUS GENOME

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Epstein-Barr virus is one of five distinct herpesviruses which afflict the human population and is the causative agent of infectious mononucleosis. The virus is carried as a latent infection by 80% of adults and has been implicated in two human neoplasias, nasopharyngeal carcinoma and Burkitt's lymphoma. In vitro, Epstein-Barr virus induces transformation of human and simian leukocytes into lymphoblasts with unlimited growth potential. The goals of this research program are to identify those EBV DNA sequences and viral RNA transcripts which are required for the induction and maintenance of the transformed lymphoblastoid phenotype and to obtain a deeper understanding of the regulation of expression of Epstein-Barr virus genetic information. The experiments will utilize restriction endonuclease analyses and electron microscope techniques to study the structure of EBV DNA and DNA sequence variations between transforming and nontransforming EBV isolates. A variety of DNA-DNA and DNA-RNA hybridization techniques will be employed in analysis of viral RNA synthesis and post-transcriptional processing in restricted, abortively infected and productively infected lymphoblastoid cell lines. Identification of 'transforming genes' and an increased knowledge of the mechanisms which control the synthesis of their RNA transcripts would facilitate an understanding of the events involved in oncogenic cellular transformation.

53. CHARACTERIZATION OF INTRACELLULAR EBV DNA FORMS

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The primary aims of the proposed project are: 1) to characterize the species of latent Epstein-Barr virus (EBV) DNA present in tissues of infectious mononucleosis (IM), Burkitt's lymphoma (BL), and nasopharyngeal carcinoma (NPC), 2) to determine the possible relation of episomal and virus DNA in producer cell lines, nonvirus producing cell lines, and hybrid cell lines, as well as the DNA of P3HR-1 virus during superinfection, 3) to determine whether host cell DNA sequences are present in episomal and/or linear virus DNA and, if so, to characterize these sequence, and 4) to determine the possible role(s) of the EBV nuclear antigen (EBNA) as a DNA binding protein in the above systems.

Virus DNA, as well as episomal and cellular DNA, will be isolated and purified according to standard techniques. Appropriate test DNAs will subsequently be cleaved with restriction enzymes and the fragments isolated. Hybridization experiments will then be employed to determine the origin of intracellular EBV DNA forms. In addition, ultracentrifugational techniques will be employed to further characterize the EBV DNA species present in virus transformed and infected cells.

Studies of the above type should aid in understanding the relationship of EBV and the oncogenic process as it applies to human systems.

54. EB VIRUS DNA CONTENT OF LYMPHOCYTES AND LYMPHOBLASTOSIS (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

55. STRUCTURAL ORGANIZATION OF HUMAN HERPESVIRUS GENOMES

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The herpesviruses are the largest and most complex of the known oncogenic DNA viruses. Amongst the human herpesviruses, cytomegalovirus, Epstein-Barr virus and Varicella-zoster virus are the most important but least understood and most difficult to study. Most adults are seropositive for several of these viruses, and usually harbor them as long-term latent infections in certain neurons or lymphocytes. CMV and VZV can occasionally cause serious clinical disease especially as primary infections in the young or when reactivated in immunodeficient patients, while EBV has been associated with infectious mononucleosis, Burkitt's lymphoma and nasopharyngeal carcinoma. Although they are all classified in the same group as herpes simplex virus, we find no detectable DNA sequence homology amongst the genomes of HSV, CMV or EBV by sensitive blot hybridization tests. Furthermore, the size and structural organization of their DNA molecules differ markedly from one another. However, these genomes are similar to the extent that all contain various combinations and arrangements of inverted duplications or tandem repeat sequences. A precise description and comparison of the location, structure and sequence organization of these repeated regions in EBV, VZV and two subgroups of CMV is the subject of this study. In HSV the inverted repeats are known to encode important regulatory functions expressed at the earliest stages of infection. From a detailed comparison of the structural organization of these genomes we hope to gain

insights into: 1) The significance and function of the various tandem and inverted repetitive DNA sequences encountered both in herpesviruses and in the genomes of eukaryotic cells in general; 2) the evolutionary divergence of the herpesviruses to produce the different tissue trophisms etc. that lead to diverse disease states and 3) the signals and mechanisms used by herpesviruses to alter and regulate gene expression in the infected host cells.

56. BIOMOLECULAR RELATIONSHIP OF HERPES VIRUS AND CANCER

Lindahl, T., Goteborgs Universitet, School of Medicine, *411 Vasaparken, S40010 Goteborg, Sweden*

OBJECTIVES: Prepare EBV DNA from virus particles. Isolate circular EBV DNA from transformed cells. Characterize circular EBV DNA from different sources. Transform circular EBV DNA molecules. Transform fragments of EBV DNA. Make probes for detecting EBV DNA.

57. DNA VIRUS GENOMES, ONCOGENESIS AND LATENCY

Pagano, J. S., Shaw, J., Bachenheimer, S., Wertz, G., Lin, J. C., Griffith, J. D., Freedlender, E., Pledger, W. J., Lockwood, A. H., University of North Carolina, Chapel Hill Campus, School of Medicine, Dept. of Medicine, *Manning Dr., Chapel Hill, North Carolina*, 27514, United States of America

The project has been expanded by the addition of a number of new faculty members and projects sponsored by the Cancer Research Center. The project involves primarily research with the Epstein-Barr virus and other herpes group viruses; it is the major source of support for EBV research at Chapel Hill. The schematic interest of the project is in persistent virus infections viewed from molecular and pathogenetic standpoints. The project concentrates on studies of the EBV DNA content of human malignant tissues against a background of basic studies of the EBV episome. EBV and cytomegalovirus chromatin structures are being investigated, as are EBV cell surface receptors and lymphocyte growth control mechanisms. It also includes projects involving study of pathobiologic mechanisms, cell-mediated immunity to EBV infections, and viral enzymology as related to antiviral substances. Core facilities that benefit the project are Tissue Culture with Virus Containment and Glassware Washing and Electron Microscopy.

58. VIRAL ONCOLOGY RESEARCH CENTER PROGRAM

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The summary for this project is under entry no. 11.

59. STRUCTURAL BASIS FOR REGULATION OF DNA VIRUS GENE EXPRESSION

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This is part of a broader project. A summary of this subproject is not available.

60. ANATOMY OF EBV DNA

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This is part of a broader project. A summary of this subproject is not available.

61. STRUCTURE OF HERPESVIRUS GENOMES

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This is part of a broader project. A summary of this subproject is not available.

62. GENE ORDER AND REGULATION OF GENE EXPRESSION

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The summary for this project is under entry no. 15.

63. MAPPING OF FUNCTION IN EBV DNA

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This is part of a broader project. A summary of this subproject is not available.

64. MAPPING OF VIRAL FUNCTIONS

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The summary for this project is under entry no. 18.

65. MOLECULAR BASIS OF GENETIC RECOMBINATION (BACTERIA, VIRUSES)

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This is part of a broader project. A summary of this subproject is not available.

66. MAPPING OF VIRAL FUNCTIONS BY ANALYSIS OF DEFECTIVE VIRUSES

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The summary for this project is under entry no. 16.

67. MAPPING OF VIRAL FUNCTIONS BY IN VITRO PROTEIN SYNTHESIS

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The summary for this project is under entry no. 17.

2. VIRUS REPLICATION

68. DNA POLYMERASES OF RNA AND DNA TUMOR VIRUSES IN HUMAN CANCER CELLS

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One of the areas of investigation that we have been interested in involves obtaining evidence for virus etiology of human cancer. We examine samples from patients with different kinds of malignancies for virus markers such as reverse transcriptase of RNA tumor viruses and a DNA polymerase specific to herpes viruses including Epstein-Barr virus (EBV). We have detected the reverse transcriptase activity in approximately 25% of the leukemic samples we examined thus far; recently, we detected in a neck mass of a patient with American Burkitt's lymphoma a DNA polymerase which resembles in its properties the EBV specific DNA polymerase. To determine whether there is any correlation between the appearance of the viral enzymes and the type and stage of malignancy and treatment, we continue to examine more samples from patients with cancer for the viral DNA polymerases. In addition, the cellular DNA polymerases alpha, beta and gamma and virus specific DNA polymerases, if present, are being purified from human cancer samples as well as from cell lines derived from patients with Burkitt's lymphoma for a qualitative and quantitative comparison with the polymerases of other eukaryotic systems. We are also investigating the mode of action of certain antitumor and anti-herpes virus agents.

69. EBV DNA SYNTHESIS IN TRANSFORMED LYMPHOCYTES

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Our laboratory studies Epstein-Barr viral (EBV) DNA replication. We have isolated and characterized a number of virally induced or associated DNA processing enzymes. An intracellular DNA polymerase and an exonuclease have been associated with productive viral DNA replication and are similar to herpes virus-induced enzymes reported in other cell systems. An EB-virion-associated DNA polymerase has been shown to have a repair-type function that is essential early in the replication

process. An intracellular endonuclease apparently creates gaps in the viral DNA, and may be a representative of a new class of mammalian endonucleases. We are currently tying our knowledge of these DNA processing enzymes in with studies of replicating viral DNA. We are isolating plasmid and linear EBV DNA replicative intermediates from drug-treated cells. Our goal is to determine the roles of the various enzymes described above in the generation of the various DNA forms observed, using DNA sizing, DNA binding and electron microscopic techniques.

70. MOLECULAR BIOLOGY AND GENETICS OF TUMOR VIRUSES

Temin, H. M., Mertz, J. E., Risser, R. G., Sugden, B., University of Wisconsin, Madison Campus, Medical School, Dept. of Oncology, *307 N. Charles St., Madison, Wisconsin*, 53706, United States of America

We are studying the molecular biology and genetics of avian and murine RNA tumor viruses and of the primate DNA tumor viruses simian virus 40 and Epstein-Barr virus. We are especially interested in the DNA genomes of these viruses and problems related to mechanisms and sites of integration, control of transcription, and mechanisms of transformation by the virus genomes.

Dr. Temin is studying the mechanisms of formation, integration, and control of expression of the provirus of avian retroviruses and their mechanism of cell killing. Dr. Mertz is studying the factors controlling transcription of the simian virus 40 genome and the viral functions involved in transforming normal cells to a tumorigenic state. Dr. Risser is studying the genetic transmission of endogenous murine leukemia viruses, modifying genes affecting the production of virus from these endogenous genomes, and the relationship between viral and H-2 antigens. Dr. Sugden is studying the molecular biology of Epstein-Barr virus. He is characterizing the viral genome in cells transformed by different numbers of virus particles and determining the viral functions responsible for transformation.

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3. VIRUS-HOST INTERACTIONS

71. GENETICS OF EPSTEIN-BARR VIRUS-HOST CELL INTERACTION - CELLULAR CONTROL MECHANISMS, CHROMOSOME ABERRATIONS AND MALIGNANCY

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OBJECTIVE: To characterize cellular controls and Epstein-Barr virus (EBV) expression and determine its relation to differentiation and transformation in human B-lymphocytes and other cell types.

APPROACH: Cellular control mechanisms involved in EBV gene expression are analyzed in somatic cell hybrids between different Burkitt's lymphoma (BL)-derived cell lines, and also in hybrids of BL cells and other cell lines derived from the human hematopoietic cell lineage. The relation between presence of the EBV genome, specific human chromosomes and malignant phenotype is analyzed in hybrids between BL cells and non-malignant mouse cells. The involvement of specific chromosomal aberrations (e.g., the BL t(8;14) translocation) and malignant phenotype is studied in lymphoblastoid cell lines (LCL) with changed characteristics.

An alternative approach is microinjection of EBV viral particles, isolated EBV specified proteins (such as the nuclear antigen EBNA), and EBV-DNA.

PROGRESS: The controls affecting EBV lytic cycle antigen (EA, VCA) expression are different in various BL-derived lines. In cell hybrids derived from different BL lines, producer (EA positive VCA positive) status dominates non-producer status (EA positive VCA negative or EA negative VCA negative). The block in abortive producer lines (EA positive VCA negative) is complemented also after fusion of abortive producers with EBV negative BL cells. The inducibility of EA (by IUdR, TPA or butyrate) is extinguished completely or partially after fusion of primitive human leukemic cells (K562 cells) with different BL cell lines.

EBNA positive hybrid cells were obtained after fusion of BL biopsy cells with mouse cells non-tumorigenic in BALB/c nude mice. The tumor-derived hybrid cells were EBNA and EBV genome negative (1 copy/cell).

Attempts to correlate specific chromosomal aberrations with increased agarose cloning of LCL cells have been unsuccessful so far; no evidence has been found for involvement of the t(8;14) marker.

In microinjection experiments, EBNA has been observed to stimulate DNA synthesis in quiescent cells. Microinjection of EBV into non-lymphoid cells allows EBNA expression.

72. EBV INTERACTION WITH LYMPHOBLASTS IN VITRO AND IN VIVO

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The specific objectives of this research grant are: (1) To biochemically transform LTK negative cells with EBV DNA; (2) to determine the complexity of EBV DNA in LTK positive transformants; (3) to analyze the structure, sequence arrangement and location of viral DNA in LTK positive transformants; (4) to analyze EBV RNA in the transformants; (5) to develop a lymphocyte system for TK transformation; (6) to apply similar techniques to the analysis of cells transformed with specific fragments of EBV DNA.

BIBLIOGRAPHIC REFERENCES: Given, D., Yee, D., Griem, K., and Kieff, E. DNA of Epstein-Barr Virus. V. Direct Repeats of the Ends of EBV DNA. J. Virol. 30:852-862, 1979. Dambaugh, T., Beisel, C., Hummel, M., King, W., Fennewald, S., Cheung, A., Heller, M., Raab-Traub, N., and Kieff, E. EBV DNA VII: Molecular cloning and detailed mapping of EBV (B95-8) DNA. Proceedings of the National Academy of Sciences, 1980, in press.

73. EXPRESSION OF EPSTEIN-BARR VIRUS IN HYBRID CELLS

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Studies on a cellular extract (D98) have continued. We have found that the D98 extract can inhibit EBV DNA synthesis by approximately 1/3 in superinfected cells as compared to the control. The inhibitory factor is sensitive to trypsin but not DNase or RNase. In addition, the inhibitory portion of the extract is nondialyzable and has a molecular weight of more than 10,000. Herpes simplex type 1 and type 2 are not inhibited by the D98 extract and no evidence associating this factor(s) with an interferon-like protein has been found.

We are studying the molecular events associated with the replication of HR-1 EBV in EBV genome positive and genome negative lymphoblastoid cell lines. Both cell types can be superinfected, but thus far, only transforming virus has been obtained from superinfected Raji cells. EBV DNA synthesis was observed in virus genome negative Ramos cells after superinfection; thus endogenous EBV DNA is not required for virus replication. The Eco R1 and Sma I restriction enzyme patterns of EBV DNA are similar in superinfected genome positive and negative nonproducer cell lines.

Studies on early and late transcription of the EBV genome have been started using control and phosphonoacetic acid treated infected cells and potassium iodide (KI) density gradients. Evidence has been found supporting temporal regulation of EBV transcription during lytic infection. EBV DNA synthesized early after infection showed a higher S value than linear 55S EBV DNA. At late stage, homogeneous unit length DNA was detected.

BIBLIOGRAPHIC REFERENCES: Glaser, R. Yajima, Y. The interaction of non-transforming Epstein-Barr virus (HR-1 EBV) with EBV positive and negative lymphoblastoid cell lines. Fourth Herpesvirus Workshop, Cold Spring Harbor, New York, 1979. p. 145. Cheng, Y.-C., Chen, J.Y., Hoffmann, P.J. and Glaser, R. Studies on the activity of DNase, DNA polymer-

ase, and thymidine kinase associated with EBV replication. Fourth Herpesvirus Workshop, Cold Spring Harbor, New York, 1979. p. 119.

4. CELLULAR TRANSFORMATION

74. MECHANISM OF TRANSFORMATION OF HUMAN CELLS WITH THE EPSTEIN-BARR VIRUS

AdamsLindahl, A., Goteborgs Universitet, Institutionen for Medicinsk Mikrobiologi, Dept. of Virology, *41346 Goteborg, Sweden*

The Epstein-Barr virus (EBV) is implicated as one of the causative agents of two forms of human cancer, poorly differentiated nasopharyngeal carcinoma and Burkitt's lymphoma. EBV is a common virus endogenous to man, and most individuals become life long carriers of the viral genome following either a subclinical infection or infectious mononucleosis. We are characterizing the EBV genomes and their expression in latently infected and transformed cells in order to understand at a molecular level the role of this virus in human cancer.

Most of the EBV genomes in virus transformed cells have a circular structure and can be isolated in pure form. To determine if EBV subtypes are associated with the various diseases, the virus genomes from cells of infectious mononucleosis, Burkitt's lymphoma and nasopharyngeal carcinoma are being characterized. The EBV genomes of most isolates are of a homogeneous size of 100 million molecular weight and these are being characterized in more detail by restriction endonuclease cleavage analysis. The EcoRI cleavage patterns of 23 isolates have been determined and the regions of greatest variability defined.

No in vitro lytic system is presently available for EBV and it is thus not possible to isolate virus mutants capable of inducing a temperature sensitive transformed phenotype as a means to define transforming functions. We are instead attempting to transfect cord blood lymphocytes with circular EBV DNA molecules. When conditions for transfection are defined we intend to specifically mutate, in vitro, those regions of the EBV DNA known to be expressed in transformed cells and determine the effect of these mutations on the transformed phenotype.

75. TRANSFORMATION OF LYMPHOCYTES BY EPSTEIN-BARR VIRUS

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The objective of this program is to understand the mechanisms by which Epstein-Barr virus transforms human lymphocytes. Related objectives include an analysis of viral functions in human Burkitt's tumor tissue and studies of the molecular epidemiology of Epstein-Barr virus. Proposed projects include: (1) Studies of the structure of viral DNA, (2) analysis of whether viral DNA is integrated into cellular DNA in transformed cells, (3) mapping of proteins encoded by viral DNA in transformed cells, (4) studies of the synthesis and processing of viral RNA in transformed cells and Burkitt's tumor tissue, (5) determination of whether there are specific binding site(s) in EBV DNA for EBNA, and (6) analyses of viral protein in transformed cells.

76. TRANSFORMATION OF HUMAN LYMPHOCYTES BY EPSTEIN-BARR VIRUS

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The long-term goal of our research is to identify the gene products that Epstein-Barr virus (EBV) contributes to transform B-lymphocytes. We are studying EBV with six varying approaches. They are: 1. Characterization of viral DNA isolated from virions and from transformed cells; 2) measurement of the target size of the viral information required to initiate and maintain the transformed state; 3) development of a method to transform lymphocytes with viral DNA; 4) selection of mutants of EBV that render the transformed cell temperature-sensitive for multiplication; 5) determination of both the amount of EBV in the saliva and number of peripheral leukocytes that release EBV in patients convalescing from infectious mononucleosis; and 6) selection of recombinants between EBV and simian virus 40 or between EBV and herpesvirus saimiri that are host-ranges mutants of EBV. Characterization of the viral DNA may permit our using defined fragments of it either to transform cells or to complement specific defects in viral mutants which render transformed cells temperature-sensitive for multiplication. The next three approaches are intended to define the amount of viral information required to induce and maintain the transformed state. The last two experiments may circumvent the problems engendered by the lack of a productive host cell for EBV.

77. STUDIES OF EPSTEIN-BARR VIRUS

Miller, I. G., Yale University, School of Medicine, Dept. of Epidemiology & Public Health, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

We propose to study several problems which bear on the mechanism of in vitro transformation of normal human lymphocytes into continuous lymphoblastoid cell lines. We shall examine the sequence of early events in transformation of purified resting B lymphocytes by Epstein-Barr virus. In particular we shall attempt to relate in time amplification of viral DNA to stimulation of cellular DNA synthesis, the appearance of EB nuclear antigen, and the first doublings of transformed cells. We shall study clonal transformants of different subclasses of human B lymphocytes in an effort to determine whether the size or physiologic state of the cell at the time of its encounter with EBV influences integration of the genome and the proportion of the genome which becomes stably associated with the cell. We shall attempt to determine whether DNA tumor viruses other than EBV can 'immortalize' human lymphocytes. Our investigations will include SV40, BK virus, herpes simplex type 1 and cytomegalovirus. We will study presently available techniques and make efforts to develop new methods to infect human lymphocytes with biologically active DNA from the papovaviruses and the herpesviruses. We shall determine whether viral multiplication, transformation or viral DNA replication occurs following addition of infectious DNA to lymphocytes. Following preliminary efforts with these well characterized DNAs we plan attempts to transform lymphocytes with EBV DNA and, if successful, with specific EBV DNA fragments.

78. EXPRESSION OF EBV DNA IN LYMPHOCYTES

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Infection of lymphocytes with Epstein-Barr virus can result in a state of partial expression of the EBV genome (termed restringent infection) in which cells are 'transformed' to capability for continued multiplication, in vitro, and contain a new intranuclear antigen, EBNA, but antigens associated with abortive and productive virus infection are not produced. The purpose of this research program is to identify the part of the viral genome functioning in the 'transformed' cell and to elucidate the biochemical mechanisms involved in control of expression of EBV DNA. Our plan is to: 1. Map viral RNA in restringently infected, continuous lymphoblastoid cell lines obtained by transforming normal lymphocytes with EBV in vitro; 2. study the structure of viral messenger RNA in Burkitt's tumor tissue; 3. study the structure and processing of viral RNA in cells biochemically (thymidine kinase) and growth transformed by (fragments of) EBV DNA; 4. study the structure of the EBNA binding site in EBV DNA and the effect of EBNA on transcription of EBV DNA; and 5. study the polypeptides made in transformed, restringently infected cells.

79. EPSTEIN-BARR VIRUS AND NASOPHARYNGEAL CARCINOMA

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OBJECTIVES: Studies on the infection by Epstein-Barr virus (EBV) of normal human epithelial cells, particularly nasopharyngeal cells; cellular hybridization studies with an HR-1 cell line containing one EBV genome per cell; studies on the transformation of normal human primary epithelial cells with EBV by a nuclear exchange procedure; studies on in vivo cell fusion of EBV genome positive lymphoid cells and nasopharyngeal cells.

80. A NEW CLASS OF EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN

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We have discovered that the Class II Epstein-Barr virus nuclear antigen (Class II EBNA), which is tightly bound to the DNA, is antigenically different from the classical EBNA which we have termed Class I EBNA and, more importantly, is related to a histone or histone-like protein which appears to be part of the EBNA complex. We now desire to purify the antigen ('protein?') and characterize its composition. We plan to compare its cell type specificity, i.e., determine whether or not it exists in EBV uninfected cells (BJAB). Eventually we wish to prepare specific antisera against this 'protein' and assess its effect on chromatin and DNA structure and template capacity (for RNA synthesis) and determine any DNA sequence specificity.

81. IMMUNOLOGICAL AND MOLECULAR HYBRIDIZATION STUDIES OF NASOPHARYNGEAL CARCINOMA

Ablashi, D. V., Aaronson, S., O'Conor, G., Levine, P., Prasad, U., Bengali, Z., Armstrong, G., Allouache, A., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Cellular & Molecular Biology, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

Epstein-Barr virus (EBV) is considered a major factor in nasopharyngeal carcinoma (NPC). The study aims to further assess the role of EBV in terms of etiology, diagnosis/prognosis and other environmental and genetic factors essential in defining the populations and individuals at risk. Studies include EBV-serology (humoral and cell-mediated), virology (attempts to isolate EBV and to compare it with known EBV isolates) and attempts to propagate epithelial cells from biopsies, examination of the HLA profile of cases, detection and examination of EBV genome expression in biopsy material by nucleic acid hybridization, review of the histopathology of all such cases and an attempt to prepare somatic cell hybrids to detect EBV receptors. Malaysia has a high incidence of NPC and has subpopulations consisting of Malayans, Indians and Chinese, whereas West Germany and the USA are low NPC incidence areas. However, in the USA, NPC cases include Chinese, white and black populations and Alaskan Eskimos. In Algeria NPC ranks as the number four cancer in males. There are also two age peaks in this population. Since NPC occurring at a young age has been observed in the black population in some southern states of the USA, Algerian NPC would provide an excellent comparison. Histopathological slides from West German and American NPC cases have been circulated for review to resolve differences in interpretation. Examination of approximately 14 cases of German NPC and more than 30 cases from Algeria suggests that antibodies to EBV antigens are much higher in Algerian cases. Data also show that antibodies to EBV are higher in American than in West German NPC. Attempts to propagate epithelial cells from NPC biopsies in vitro were unsuccessful.

82. MOLECULAR BASIS OF VIRAL ONCOGENESIS

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The summary for this project is under entry no. 12.

83. MOLECULAR BIOLOGY AND GENETICS OF TUMOR VIRUSES

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The summary for this project is under entry no. 70.

C. OTHER HERPESVIRUSES

1. STRUCTURE AND GENOMIC ORGANIZATION

84. COMPARATIVE STRUCTURE AND BIOLOGY OF HERPESVIRUS GENOMES

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Three equine herpesviruses (EHV-1,2,3) that differ in biological and biochemical properties are being studied. EHV-1 causes systematic infection that may be abortigenic; EHV-2 is a cytomegalovirus associated with several diseases; EHV-3 causes a venereal disease. EHV-1 has oncogenic potential as both inactivated standard virus as well as live virus preparations rich in defective interfering particles oncogenically transform hamster (and mouse) embryo cells. Despite differences in biological properties, preliminary studies have indicated that the genomes of these herpesviruses share DNA sequences. However, nothing is known of the molecular structure of the genome or the nature of the shared sequences. These studies are designed to elucidate and compare the molecular anatomy of the genomes of these three related herpesviruses by restriction enzyme mapping techniques and to determine the nature, location and possibly some function(s) of type-shared DNA sequences. Of great importance are the studies to determine whether viral sequences present in EHV-1 transformed cells and tumor cells and sequences reiterated in the genome of DI particles (studies supported by other funding) are those shared by EHV-2 and/or EHV-3.

85. STRUCTURAL ORGANIZATION OF HUMAN HERPESVIRUS GENOMES

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The summary for this project is under entry no. 55.

86. STRUCTURE OF HERPESVIRUS GENOMES

Summers, W. C., Yale University, School of Medicine, Dept. of Human Genetics, *333 Cedar St., New Haven, Connecticut,* 06510, United States of America

The summary for this project is under entry no. 61.

87. DNA VIRUS GENOMES, ONCOGENESIS AND LATENCY

Pagano, J. S., Shaw, J., Bachenheimer, S., Wertz, G., Lin, J. C., Griffith, J. D., Freedlender, E., Pledger, W. J., Lockwood, A. H., University of North Carolina, Chapel Hill Campus, School of Medicine, Dept. of Medicine, *Manning Dr., Chapel Hill, North Carolina,* 27514, United States of America

The summary for this project is under entry no. 57.

88. MOLECULAR BIOLOGY OF HUMAN CYTOMEGALOVIRUS

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The human cytomegaloviruses (CMV) are ubiquitous in human populations and are associated with numerous diseases, some of which are fatal. I am presently studying the molecular biology of human CMV infections, including the molecular organization of the human CMV genome, the interaction of CMV with cells during permissive and nonpermissive infections, and the oncogenic transformation of cells by CMV. In particular, I am focusing on the nature and association of the viral DNA with the cellular genome and on the regulation and characteristics of the virus-specific transcription and translation products. For these studies, I use a wide variety of molecular techniques, including physico-chemical analyses of DNA, fragmentation of DNA with restriction endonucleases, the DNA transfer procedure of Southern, the RNA transfer procedure of Alwine et al., nucleic acid hybridization with highly defined reagents, the immunoprecipitation of viral proteins from infected cells, and the cell-free translation of viral RNA. The major objectives of my research are 1) to define thoroughly the molecular biology of human CMV infections in permissive cells, 2) to characterize the molecular events occurring during nonpermissive infections and their relationship to possible mechanisms of latency, and 3) to determine the oncogenic potential of human CMV.

BIBLIOGRAPHIC REFERENCES: D.H. Spector, K. Smith, T. Padgett, P. McCombe, D. Roulland-Dussoix, C. Moscovici, H.E. Varmus, and J.M. Bishop (1978). Uninfected avian cells contain RNA related to the transforming gene of avian sarcoma viruses. Cell, 13, 371-380.

89. ANALYSIS OF HUMAN CYTOMEGALOVIRUSES

Huang, E., Mar, E., Baskar, J. F., University of North Carolina, Chapel Hill Campus, School of Medicine, Dept. of Medicine, *Manning Dr., Chapel Hill, North Carolina,* 27514, United States of America

GOALS SET FOR THE CURRENT YEAR: 1. To continue efforts in analysis of CMV DNA by various restriction endonucleases. 2. To continue the study of genetic variability of human cytomegalovirus both in vitro and in vivo. 3. To continue efforts toward establishment of the genetic relatedness of CMV and other herpes group viruses. 4. To study the mode of CMV transmission and the effect of CMV infection on embryonic development. 5. To continue efforts toward location of viral genome, viral antigen and susceptible cell type by nucleic acid hybridization and various immunological techniques. 6. To continue the comparative study of herpes group virus-induced DNA polymerases.

The project's overall objectives are (a) to study the molecular biology of human cytomegalovirus (CMV), (b) to elucidate the mode of viral transmission in interuterine infection and (c) to understand the biopathology of CMV infection.

BIBLIOGRAPHIC REFERENCES: Huang, E-S., Kilpatrick, B.A., Lakeman, A., and Alford, C.A. 1978. Genetic analysis of a peculiar cytomegalovirus-like agent isolated from human brain. J. Virology 26: 718-723. Mar, E-C. and E-S. Huang. 1979. Comparative study of herpes group virus-induced DNA polymerases. Interovirology (in press).

90. HOST-DEPENDENT HERPESVIRUS MUTANTS

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This is part of a broader project. A summary of this subproject is not available.

91. STRUCTURAL BASIS FOR REGULATION OF DNA VIRUS GENE EXPRESSION

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The summary for this project is under entry no. 59.

2. VIRUS REPLICATION

92. STIMULATION OF DNA SYNTHESIS IN CELLS INFECTED BY HERPES VIRUSES

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The summary for this project is under entry no. 26.

93. CYTOMEGALOVIRUS TRANSCRIPTION AND TRANSLATION

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My research on human cytomegalovirus (CMV) is designed to develop an understanding of the regulation of a complicated DNA virus genome. This virus can replicate in cells of human origin or remain latent in these cells for extended periods of time. The problem is to be approached by characterizing the transcription of the CMV genome in permissive cells that permit viral DNA replication and infectious virus production and in nonpermissive cells that allow early viral gene expression but no viral DNA replication or detectable late gene expression. The nonpermissive cell is being used as an example of a cell that favors latency of the CMV genome. The origin of viral transcripts synthesized in permissive and nonpermissive cells will be mapped on the viral genome. Host- or virus-induced proteins that may be regulatory proteins because they influence viral gene transcription, bind to the viral DNA, covalently link to viral DNA, or interact with host cell chromatin will be characterized to increase understanding of the regulation of viral replication and the interaction of viral gene products with the host cell. I also propose to initiate an analysis of the genetic organization of the CMV genome by translating in vitro mRNAs from defined regions of the viral genome. The organization of the CMV genome and its replication in permissive and nonpermissive cells will be related to the latent or persistent infectious potential of the virus in humans.

94. THE ROLE OF HOST CELL DNA SYNTHESIS IN THE REPLICATION OF CYTOMEGALOVIRUS

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This is part of a broader project. A summary of this subproject is not available.

95. HERPES VIRUS DNA REPLICATION

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This is part of a broader project. A summary of this subproject is not available.

96. STRUCTURAL ORGANIZATION OF HUMAN HERPESVIRUS GENOMES

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The summary for this project is under entry no. 55.

3. VIRUS-HOST INTERACTIONS

97. LATENCY IN HERPES VIRUS INFECTION OF CELLS AND TISSUES

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The summary for this project is under entry no. 31.

98. CYTOMEGALOVIRUSES AND HUMAN MALIGNANCY

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The overall objective of this project is to investigate the causal association between human cytomegalovirus (human CMV) and various human malignancies, especially the virus and cell interactions that might bear upon the mechanism of herpesvirus oncogenesis. The long-term objectives are directed by the following approaches: a) Detection of the existence of human CMV genome in various cancer tissues by nucleic acid hybridization techniques. b) Observation of virus and cell interaction by transforming various mammalian cells with viruses isolated from various tumor tissues and detection of virus genome and virus message in transformed cells. c) Genetic analysis of human CMV strains isolated from various clinical manifestations. d) Designation of the viral DNA restriction fragment which is responsible for the virus oncogenesis.

99. MOLECULAR BASIS OF GENETIC RECOMBINATION (BACTERIA, VIRUSES)

Radding, C. M., Yale University, School of Medicine, Dept. of Human Genetics, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

The summary for this project is under entry no. 65.

4. CELLULAR TRANSFORMATION

100. MECHANISMS OF TRANSFORMATION BY ONCOGENIC VIRUSES

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Induction: The interaction of the virus genome with the host cell genome in transformed cells is being investigated. Specifically, analyses will be made of the early events in induction utilizing chemicals and radiation. The mechanism of viral derepression and excision of the viral genome, in particular the effects of breaks in the host cell DNA on virus induction will be examined. The properties of excised viral DNA, the kinetics of virus DNA synthesis, viral transcription, and the factors involved in permissivity will be proved by comparing induction in various clones of transformed hamster cells which are heterogeneous with respect to their inducibility.

Cell surface: The mechanism(s) responsible for the phenotype of the transformed cell will be analyzed with emphasis on the role of cellular proteases. The process regulating secretion will be studied since continuous secretion of proteases occurs

from transformed cells while secretion of proteases, which occurs in normal growing cells, ceases in normal confluent cells. A relationship between secretion of cellular proteases and loss of proteins from the transformed cell surface will be sought.

Antibody producing transformed cell lines: Studies whereby antibody producing rabbit lymphoreticular cells have been transformed by SV40 and Herpesvirus saimiri and converted to antibody secreting autonomous cell lines will be continued. We plan to produce an autonomous human lymphoreticular cell line secreting antibody which could be utilized for diagnosis and therapy of human diseases by sensitizing human lymphoid cells against a relevant antigen such as digoxin and subsequently transforming these cells with a B lymphocyte tropic virus such as EBV.

BIBLIOGRAPHIC REFERENCES: Black, P.H. Shedding from the cell surface of normal and cancer cells. Adv. Cancer Res. 32:75-199, 1980. Zamansky, G.B., Kleinman, L.F., Black, P.H. and Kaplan, J.C. Reactivation of herpes simplex virus in a cell line inducible for simian virus 40 synthesis. Mutation Res. 70:1-9, 1980.

101. HERPESVIRUS-INDUCED MALIGNANCY

St. Jeor, S. C., Hall, M., Winicov, I., University of Nevada, Reno Campus, School of Medical Sciences, Dept. of Microbiology, *Reno, Nevada,* 89507, United States of America

The overall goal of this research proposal is to develop an understanding of the molecular biology of human cytomegalovirus (CMV) and use this information to explain the mechanism of cell transformation and latency by CMV. Although human CMV is suspected of having oncogenic potential because of the low rate of cell transformation, it is difficult to study the events involved in virus transformation. We intend to use cells productively and abortively infected with CMV to examine the following areas: a) Control of virus gene expression during productive infection. b) Control of gene expression during latent virus infections. c) Maintenance of CMV genetic information during productive and latent infections. d) Function of cell macromolecular synthesis during CMV replication. The control of gene expression will be studied by examining transcription of virus mRNA in an attempt to understand the sequential control of virus gene expression. Factors to be examined include transcription, post-transcriptional processing and transport of mRNA. Classes of virus mRNA will be identified and the region of the genome from which transcription is occurring identified. Latency will be studied and an attempt made to relate this to transformation. Problems to be investigated include: a) Gene expression during latency, b) maintenance of CMV genetic information during latency, and c) the potential for using its mutants to examine latency and cell transformation. The induction of cell macromolecular synthesis will be studied in an attempt to further understand its function in virus replication.

BIBLIOGRAPHIC REFERENCES: St. Jeor, S.C., Chua, C.C., and Ziegler, J.L. Expression of cytomegalovirus antigens in productive and abortive infections. Herpesvirus Workshop, St. John's College, Cambridge, England. August 20-25, 1978, p. 82. Tocci, J.J., and St. Jeor, S. The susceptibility of lymphoblastoid cells to infection with human cytomegalovirus. Infect. Immun., 23 418 Feb., 1979.

102. MOLECULAR BIOLOGY OF VARICELLA-ZOSTER VIRUS

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The varicella-zoster virus (VZV) causes the most widespread childhood infection not yet amenable to vaccine control. It is associated with significant morbidity and mortality expecially among the immunosuppressed both during primary infection and during reactivation (shingles). Its control has been hampered by difficulties in virus propagation and purification related to its cell-associated nature in vitro.

We propose to continue to search for a more efficient propagation system employing a variety of cell lines (both fibroblastic and epithelioid) and using the appearance of CPE, VZV particles as seen in the electron microscope, and hybridizable VZV mRNA and DNA as criteria for yield. VZV nucleocapsids will be purified to provide a source of viral DNA and to eliminate host proteins from the preparation. VZV DNA will also be purified directly from infected cells using the Hirt procedure.

The site of varicella-zoster latency will be investigated by analyzing human dorsal ganglia DNA for the presence of VZV-specific nucleic acids. We will infect human neuronal cells in vitro to probe the mechanism of VZV latency. The question of VZV oncogenicity will be evaluated by continuing our in vitro transformation experiments. Varicella-zoster virus infection transforms hamster embryo cells. These cells demonstrate newly acquired VZV-specific surface antigens and Fc receptors. They produce tumors in syngeneic hamsters. We intend to quantitate the frequency of VZV-induced transformation and document the presence of VZV-specific nucleic acids in the transformed cells.

II. PAPOVAVIRUSES

A. SV-40

1. STRUCTURE AND GENOMIC ORGANIZATION

103. GENETICS AND STRUCTURE OF THE ONCOGENIC VIRUS, SV40

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The early region of SV40 codes for two proteins of 20K and 90K. Deletion mutants that eliminate the 20K protein, temperature-sensitive mutants in the 90K proteins and double mutants have been constructed. From studies with these mutants it is clear that the 20K protein is not required for viral replication. The onset of tumor appearance in hamsters inoculated with the deletion mutants is delayed, but tumors are found with high frequency. In tissue culture, the deletions affect the frequency of transformation in resting but not in growing cells. In appropriate growth factor deficient media a requirement for the 20K or for the 90K proteins for the maintenance of transformation can be demonstrated. Depending upon the cell lines used, a direct correlation between the condition of transformation and temperature-sensitivity of the resulting cell line is found following infection with mutants that are ts in the 90K protein. Analyses of the pattern of integrated SV40 in many cell lines suggest that SV40 does not integrate as a circle, but perhaps only as a tandem linear molecule. The latter suggests that SV40 may replicate by the rolling circle mode in nonpermissive infections.

BIBLIOGRAPHIC REFERENCES: C. A. F. Edwards, G. Khoury and R. G. Martin: Phosphorylation of T-antigen and control of T-antigen expression in cells transformed by wild-type and tsA mutants of SV40. J. Virol. 29,753-762, 1979.

104. THE STRUCTURE AND ASSEMBLY OF SIMIAN VIRUS 40 CHROMATIN

Bina, M., Purdue University, School of Science, Dept. of Chemistry, *Executive Bldg., West Lafayette, Indiana,* 47907, United States of America

Simian virus 40 (SV40) belongs to the DNA containing group of cancer causing viruses. During the course of infection of permissive cells, the virus stops cell division, kills the cells and produces infectious progeny. I am interested in learning about the molecular interactions that control the structure, expression and assembly of SV40 virus in permissive host cells. Previously, I have studied the mode of minichromosome formation caused by the interaction of the major chromosomal proteins (histones) and the SV40 DNA. My current goal is to elucidate the roles played by the virus coded structural proteins in folding and expression of minichromosomes.

An important approach to the study of the structure and function relationships of biological macromolecules involves using appropriate temperature sensitive (ts) mutants, and identifying how a precise change in protein structure can alter function. I will characterize two classes of SV40 ts mutants by biochemical and morphological techniques. In one series of experiments, with temperature sensitive mutants in the major virus structural protein VP1 (ts B/C mutants), the roles of histone H1 and VP1 in 'specific' higher order folding of SV40 minichromo-

somes will be determined. In a second series of experiments, with temperature sensitive mutants in the minor structural proteins VP2 and VP3 (ts D mutants), I will determine whether VP2 and VP3 regulate the expression of the tumor specific antigens. These studies will lead to a better understanding of the molecular mechanisms which regulate gene expression and control virus production.

105. SV40 VIRUS-INDUCED CARCINOGENESIS - STRUCTURE OF THE VIRAL GENOME AND THE MECHANISM OF CELLULAR TRANSFORMATION

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Our long-term goals have been, and continue to be, to: a) Define the genetic elements of the SV40 genome that are essential for the vegetative multiplication of the virus and that are responsible for cellular transformation and oncogenesis. b) Understand how these genes are expressed and regulated during vegetative growth and in transformed cells. c) Identify SV40 gene products and determine how and on what cell targets they act in transformation and oncogenesis. d) Determine how and where the viral oncogenes become stably associated with the host genome.

Presently, our principal concern is learning more about the transcription of the early region of SV40, the structure of the primary transcript(s), their processing to mature mRNAs, and the protein products these mRNAs code for. Ultimately, we want to understand how and where these viral-coded proteins promote cellular transformation. Our plan is to explore these problems in acutely infected permissive cells (primate cells) as well as in non-permissive and transformed cells.

106. THE STRUCTURE OF THE INTEGRATED VIRAL DNA IN SV40-TRANSFORMED CELLS

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This project is aimed at a characterization of the integrated viral DNA in SV40 transformed cells. The specific objective of this research is to isolate DNA fragments containing the SV40-cell DNA recombinant junction and to determine the nucleotide sequence of the recombination site. Two approaches will be employed to isolate large amounts of integrated viral DNA in covalent association with the cellular DNA sequences which flank it in the integrated state. One approach is to use fusion of permissive cells with SV40 transformed cells to rescue SV40 DNA molecules which contain host DNA substitutions. A second approach is through DEAE-mediated transfection of permissive cells with restriction endonuclease generated DNA fragments which contain linked cellular and viral DNA. Once molecules of SV40 DNA which contain host DNA have been isolated, they will be analyzed by restriction endonuclease digestion and gel electrophoresis to identify the DNA fragments which contain the junction between viral and cellular DNA. The nucleotide sequence of these fragments will be determined and compared with those of the parental DNA molecules to identify those sequences involved in the recombination event which produced viral integration.

107. STRUCTURE AND FUNCTION OF DNA TUMOR VIRUS GENOMES

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The immediate objective of the proposed research program is to relate nucleotide sequence to function in several regions of the simian virus 40 genome believed to contain regulatory functions. The first region to be studied will be the SV40 origin of DNA replication. This study will utilize previously constructed, viable variants of SV40 which contain two functional origins of DNA replication. Deletion and point mutations will be introduced in the region containing the origin of DNA replication, the mutants will be assayed for altered function, and the alteration in the nucleotide sequence will be determined. Similar studies will be carried out in regions believed to contain promotors for viral transcription, regions surrounding known 'splice' points in 'early' SV40 mRNAs, and the region which encodes the 3' noncoding segments of both 'early' and 'late' SV40 mRNAs.

BIBLIOGRAPHIC REFERENCES: Jones, N. and Shenk, T. (1978). Isolation of Deletion and Substitution Mutants of Adenovirus Type 5. Cell 13, 181-188. Shenk, T. (1978). Construction of a Viable SV40 Variant Containing Two Functional Origins of DNA Replication. Cell, in press.

108. TUMOR VIRUS CHROMATIN - STRUCTURE AND REPLICATION

Wassarman, P. M., Harvard University, Boston Campus, School of Medicine, Dept. of Biological Chemistry, *25 Shattuck St., Boston, Massachusetts*, 02115, United States of America

The objective of this research is to determine the exact relationship between the structure of chromatin and replication of DNA. This relationship is fundamental to understanding the molecular basis of mammalian cell growth, tumor virus replication, integration of viral DNA into cellular DNA, and transformation of normal cells into cancer cells.

The selection of the SV40 genome as our primary experimental system will allow us to analyze in detail a pure, homogeneous, undamaged population of chromatin. This viral genome is well characterized and can be isolated as intact replicating molecules which will permit us to study the disassembly and reassembly of chromatin during replication, as well as the role of chromatin structure in the process of discontinuous DNA synthesis.

BIBLIOGRAPHIC REFERENCES: Shelton, E., Kang, J., Wassarman, P., and DePamphilis, M. (1978) Chromatin assembly in isolated mammalian nuclei. Nucleic Acids Research, 5, 349-362.

109. ORGANIZATION AND EXPRESSION OF GENES IN VIRAL DNAS

Roberts, B. E., Miller, J. S., Jones, R., Harvard University, Boston Campus, School of Medicine, Dept. of Biological Chemistry, *25 Shattuck St., Boston, Massachusetts*, 02115, United States of America

The structural and regulatory sequences within SV40 and adenovirus 2 DNA, expressed early and late in infection, will be precisely mapped using both linked transcription-translation cell-free systems and hybrid arrested translation. DNA sequences coding for specific information will then be correlated directly with their corresponding messenger RNAs. These precise coordinates of coding and non-coding regions within DNA will be compared directly to those of the RNA splicing in order to ascertain the possible function of this process in messenger RNA maturation. Furthermore, this precise mapping will be used in the construction of experiments to study the regulation of differential gene expression during viral infections and also virally induced alterations of host cell functions.

BIBLIOGRAPHIC REFERENCES: Kronenberg, H.M., Bryan E. Roberts, and Argiris Efstratiadis (1979) The 3' noncoding region of beta-globin mRNA is not essential for in vitro translation, Nucleic Acids Research 6(1):153-165. Ricciardi, R.P., J.S. Miller and B.E. Roberts (1979) Purification and Mapping of Specific mRNAs by Hybridization-Selection and Cell-Free Translation, PNAS, 76: 4927.

110. ISOLATION AND CHARACTERIZATION OF THE GENE CODING FOR SV40 MIDDLE-T ANTIGEN

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We propose to study in detail the nature of the gene(s) encoding the middle-t antigen of the human cells transformed by simian virus 40 (SV40). The aim of the initial stages of the project will be to clone, in prokaryotic vectors, reverse transcripts of mRNA(s) which code for the antigen, and to use these clones to isolate from a library of cloned fragments of human DNA, the genomic sequences which specify the antigen. Later phases of the work include sequence analyses of the genomic and cDNA clones, and comparison of the structures of the genes and their mRNAs. In this way we shall determine whether intervening sequences are present in the natural middle-t antigen gene. This availability of well characterized clones carrying middle-t sequences will open up many future possibilities, which include: a) Obtaining expression of the gene in prokaryotes, b) studies of the mechanism by which the concentra-

tion of cellular middle-t antigen is greatly elevated in response to viral large T antigen, c) re-introducing the gene to untransformed mammalian cells.

111. SITES OF PAPOVAVIRUS GENOMES IN TRANSFORMED CELLS

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OBJECTIVES: Construct physical maps of SV40 DNA integrated in the genome of transformed cells and pinpoint the site for integration. Isolate fragments of the high molecular weight DNA from SV40-transformed cells enriched for the junction between the cell DNA and the integrated viral DNA. Determine the nucleotide sequences at the junctions between the viral and cell lines and compare these sequences in order to determine whether they share a common feature. Repeat these investigations using the DNAs from the human papovavirus, BKV, and corresponding transformed cell lines. Determine if DNA extracted from human tumors and tumor cell lines contains sequences complementary to SV40 DNA, and, if so, investigate them further to determine the nucleotide sequence at the junction between the viral and cell DNA.

112. A NOVEL FUNCTION OF THE SIMIAN VIRUS 40 GENOME

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Examination of a wide variety of simian virus 40 mutant and defective genomes shows that, with one possible exception, they all have retained, in addition to the replication origin at map position 0.67, either a portion of the viral DNA near map position 0.16 or a host DNA substitution. The requirement for the viral termination region is particularly strict in A172 cells, a human glioblastoma line which supports the lytic growth of SV40 and rapidly accumulates a high proportion of defectives, even on low multiplicity passage of the virus. This has led to the hypothesis that the termination region, or sequences substituting for it in function, is required in cis for propagation. By use of appropriate restriction enzymes, variant viral genomes lacking the termination region will be constructed and tested for viability on co-transfection with non-defective DNA. The ability of the viral termination region and of host cell sequences recovered from natural defectives to restore viability to non-propagatable variants will be examined. If the necessity of retaining the termination region is confirmed, its role in the lytic cycle will be investigated in studies of the replication and transcription of various defectives.

The differences between A172 cells and monkey cells with respect to their treatment of defectives will be examined. First, natural defectives accumulated in one cell type will be tested for their viability and competitive advantage in the other host. Second, metabolic parameters of the cells before and after infection will be measured which may be relevant to the generation and accumulation of defectives. Such cell characteristics may be generally important in determining which tissues are subject to acute and which to persistent infection by many viruses.

113. FUNCTIONS OF REGULATORY SEQUENCES IN SV40 DNA

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Simian virus 40 (SV40) is a small oncogenic virus whose DNA is capable of replication and expression within a eukaryotic environment. The complete nucleotide sequence of SV40 DNA is now available. Nearly 14% of the DNA does not code for protein. A good part of this untranslated DNA is present as a continuous segment mapping between 0.655 and 0.765 map units in the DNA. This region consists of the origin of DNA replication, three pairs of reiterated sequences and the leader sequences of the late viral messenger RNAs. Some of these sequences are believed to be involved in initiation of transcription and post-transcription processing of viral RNA molecules. The objective of the proposed research is to correlate nucleotide sequence of the viral DNA with its function in regions of the DNA involved in regulation of genome expression. Our approach for this study is to construct viral deletion mutants lacking one specific sequence at a time and to determine at what stage of genome expression the mutant becomes defective. Sequences to be examined in this way are: a) The 203 nucleotide long leader sequence of the major late viral messenger RNA (we have already constructed mutants lacking this sequence using a procedure developed by me and mapped the deletions accurately by DNA sequencing); b) sequences involved in 'splicing' of the late viral mRNAs; and c) the three sets of reiterated sequences occurring in the beginning of the late region. Emphasis will be placed on studying the pattern of RNA synthesis in mutants lacking each of these sequences to determine whether transcription or processing of the viral RNAs is affected, and, if processing is affected, the nature of unprocessed (or slowly processed) intermediates that accumulate.

114. THE MECHANISM AND CONTROL OF VIRAL AND CELLULAR GENE EXPRESSION IN SV40-TRANSFORMED CELLS

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OBJECTIVE: We wish to analyze in detail the mechanisms by which the integrated viral sequences present in SV40-transformed mouse cells are expressed, and to understand how the viral tumor antigens affect the expression of cellular genes.

APPROACH: The structure of the integrated viral DNA is being analyzed by cloning these integrated sequences in lambda vectors. Such recombinant clones are then used for restriction endonuclease mapping and DNA sequencing studies. We are also using molecular cloning techniques to investigate the mechanism of integrative recombination.

The integrated viral DNA is transcribed into mRNAs which are the templates for the synthesis of the viral tumor antigens. The structures of these mRNAs are being determined by S1 mapping and cDNA sequencing and the corresponding proteins, once identified by in vitro translation, are studied by peptide mapping. We thus hope to achieve a detailed description of how the integrated SV40 DNA is expressed.

We are also attempting to identify and isolate cellular genes that are affected by SV40-transformation with a view to investigating the interactions between viral gene products and the cellular genome. Of particular interest to us are the genes coding for fibronectin and nvT-antigen (middle T-antigen).

115. THE EFFECT OF DELETIONS ON THE SPLICING OF EARLY SV40 EARLY MESSENGER RNAS

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Viable mutants of SV40 with deletions in the region between 0.53 and 0.60 affect the synthesis of small-t antigen although large-T is unaffected (Crawford et al., Proceedings of the National Academy of Sciences USA, 1978, 75, 117). The DNA from several of these mutants has now been sequenced and we can relate the DNA sequence of the mutants with the predicted amino acid sequence of the small-t-related polypeptides which they produce (Crawford and O'Farrell, Journal of Virology, 1979, 29, 587). Some of the mutants cause a change in the reading frame so that translation continues after the deletion and terminates beyond the usual termination codon.

Three of the mutants - dl 883, dl 884 and dl 2122 - have lost one of the regions involved in splicing to produce the small-t mRNA. Little or no small-t-related polypeptide is produced in cells infected by any of these three mutants in spite of the fact that most of the region coding for small-t is unaffected. Also small-t can be synthesized from unspliced RNA since the splice in small-t mRNA occurs outside the coding region. It seems, therefore, that splicing of the small-t messenger is required for production of active messenger RNA and its transport from nucleus to cytoplasm in infected cells.

116. GENE TRANSFER BY MEANS OF DEFECTIVE VIRUSES

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OBJECTIVE: The simian virus 40 (SV40) 'early' region DNA is used as a vehicle to introduce cloned eukaryotic genes back into eukaryotic cells in order to study their expression.

APPROACH: SV40 'early' DNA was cloned in the bacterial plasmid pBR322. Such recombinant DNA retained its biological activity, since about 5% of the monkey cells treated with this DNA produced SV40 T antigen. Triple recombinants were made by cloning further genetic information into the pBR322-SV40 early molecule: sea urchin histone genes, the rabbit beta globin major gene, Xenopus histone genes. Such triple recombinant plasmids were grown in HB101 bacteria and prepared in milligram amounts in order to obtain starting material: (a) Transformation of nonpermissive cells, and (b) plasmid-like replication within permissive CV1 monkey cells.

PROGRESS: (a) Rat cells were transformed with circular 4,500 bp recombinant DNA consisting of the SV40 early region and a short segment of sea urchin histone DNA. It was found that the sea urchin DNA was co-integrated with SV40 DNA, and that after many cell divisions the original circular recombinant DNA could be recreated by fusing transformed rat cells with CV1 monkey cells (Schaffner, W., Topp, W. and Botchan, M., 1978, Alfred Benzon Symposium 13, 191-203). (b) Triple recombinant DNA of 15,000 bp exceeding SV40 wildtype DNA (5,224 bp) consisting of pBR322, SV40 early region and sea urchin histone genes was taken up by monkey cells, but unexpectedly could not replicate. The replication blocking function was found to act in cis and to reside within pBR322 DNA, since co-infecting SV40 early-sea urchin histone genes could replicate. Studies on the expression of SV40-linked genes are in progress.

117. ANALYSES OF INTEGRATION OF PAPOVAVIRUS DNA IN TRANSFORMED CELL DNA

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Experiments were initiated with two mouse cell lines, a parental line carrying at least one integrated copy of SV40 DNA per diploid equivalent of cell DNA and a subline obtained by immunoselection carrying about 0.5 copies of SV40 DNA per diploid equivalent of cell DNA. Cloned derivatives of both lines were stable. The parental line synthesized early SV40 RNA and expressed early viral antigens. The subline produced no virus-specific RNA or antigens. Electropherograms of restriction endonuclease digests of the cellular DNAs, denatured and transferred to nitrocellulose paper, were hybridized with labeled SV40 DNA to localize SV40 and demonstrated two loci of integrated SV40 DNA in each cell line. Hpa I digests of the parental cell line contained 4 fragments of SV40 DNA in common with the subline, but also contained 5 fragments of SV40 DNA absent from the subline. Other restriction enzyme digests supported the conclusion that a complex series of deletions from both loci of integrated SV40 DNA in the parental line were represented in the subline and further study was discontinued.

BIBLIOGRAPHIC REFERENCES: Israel, M.A., Martin, M.A., Takemoto, K.K., Howley, P.M., Aaronson, S.A., Solomon, D. and Khoury, G.: Evaluation of normal and neoplastic human tissue for BK virus. Virol. 90: 187-196, 1978.

118. BIOCHEMICAL MAPPING OF THE INTEGRATION SITE OF SV-40

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OBJECTIVES: Sequence approximately thirty nucleotides of cellular DNA immediately adjacent to viral DNA in substituted evolutionary variants. Clone and characterize additional variants as necessary.

119. MAPPING OF SV40 TRANSCRIPTION UNITS

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We propose to define the transcriptional organization of the genome of simian virus 40 (SV40) during productive infection of permissive cells and in transformed non-permissive cells using an ultraviolet light (UV) mapping technique developed in prokaryotic systems. Since the UV inactivation cross-section for the expression of a particular gene is proportional to the distance between the transcriptional start point (promoter) and the promoter distal end of the gene, the locations of promoters can be determined by measuring UV inactivation kinetics for gene expression.

We are measuring the UV inactivation kinetics for the synthesis of the SV40 late proteins using SDS polyacrylamide gel electrophoresis and for the late mRNAs using hybridization of RNA to restriction fragments of SV40 DNA immobilized on nitrocellulose filters. Assays for SV40 early RNA are being carried out using prior selection of the SV40 RNA on DNA-sepharose columns followed by filter hybridization. UV mapping is also being applied to the study of the expression of the adenovirus 2 late genes.

120. GENE EXPRESSION OF A SMALL DNA TUMOR VIRUS - SV40

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The specific research objectives and plan in this work are: 1. To determine, by genetic manipulation, the important control elements that regulate SV40 gene expression. 2. To construct, by biochemical methods, a series of well defined non-viable deletion mutants in the putative control segments of an SV40-hybrid virus; SV40-beta globin. 3. To determine the effects of the constructed deletion mutants on the expression and processing of the beta globin sequences thus circumventing the difficulties of studying non-viable mutants and taking advantage of the extensive molecular knowledge of the globin gene. 4. To follow transcriptional changes during defective SV40 evolution and during the establishment of persistent SV40 infections. 5. To characterize the alterations in SV40 'defectives' that abnormally 'splice' RNA.

121. MUTAGENESIS OF SPECIFIC REGIONS OF THE SV40 GENOME

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The objective of this research proposal is to saturate the genome of SV40 with point mutations for the purpose of demonstrating the functional relationships among SV40 gene products, especially those which lead to and alter malignancy. This goal will be accomplished by selectively mutagenizing specific underrepresented regions of the chromosome by taking advantage of the mode of action of certain mutagens, the mutational blocks in existing mutants, and the relative ease of genetic manipulation of SV40. In order to increase the likelihood of identifying mutations throughout the genome, temperature-sensitive, point-lethal, and host range-transformation mutants will be isolated and characterized as to phenotype and map location. The identification of point mutations in all possible regions of the SV40 genome and characterization of the alterations they produce should add significantly to our understanding of the functioning of viral information during lytic infection and transformation.

BIBLIOGRAPHIC REFERENCES: Tevethia, S.S., Greenfield, R.S., Rieder, V. and Tevethia, M.J. Biology of simian virus 40 (SV40) transplantation antigen (TrAg). IV. Inhibition by human interferon of expression of SV40 TrAg in SV40-infected monkey cells. Virology, 95: 587-592, 1979.

122. FUNCTION OF SV40 GENE PRODUCTS

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Work is continuing on the study of the possible functions of cell sequences that show homology to SV40 viral sequences. In particular we have found that the interspersed repetitive DNA consists of nucleotide stretches of about 265 bases show-

ing strong homology with one another and showing some homology with the origin of replication of papovaviruses. These DNA sequences act as templates in vitro for polymerase III transcription and we are currently examining the RNAs produced in vivo that are homologous to these sequences and testing whether they also serve some role in the initiation of DNA replication.

123. NUCLEIC ACID-PROTEIN INTERACTIONS IN THE SV40 T ANTIGEN

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I propose to investigate certain chemical properties of a primary early SV40 gene product - 'large' T antigen - and its binding to the SV40 genome. My goal will be to identify specific DNA sequences of specific 'large' T antigen binding sites on the SV40 chromosome. Two major approaches will be tried - protection of unique 5'-labeled DNA fragments against exonuclease III digestion and protection of specific residues against modification with dimethylsulfate. I wish to gain further insights into the chemical basis for the specific interactions of this protein and specific SV40 genomic loci.

124. MAPPING OF TUMOR VIRUS GENOMES IN TRANSFORMED CELLS

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It is the objective of this project: 1. To map the chromosomal site of integration of SV40 in different SV40 transformed human cell lines, 2. to establish the role of the SV40 A gene product in the proliferation of somatic cell hybrids between mouse peritoneal macrophages and SV40 transformed human cells, 3. to produce antisera for surface antigens coded by the human chromosomes carrying the SV40 genome, 4. to study the expression of SV40 T antigen in hybrids between mouse teratocarcinoma and SV40 transformed human cells, 5. to map the chromosomal site of integration of SV40 in different SV40 transformed mouse cell lines, 6. to map the transforming gene(s) of adenovirus 5 in adenovirus transformed cell lines, 7. to establish whether the genome of Epstein-Barr virus is chromosomally integrated in Burkitt's lymphoma cells derived from different patients; 8. to map the chromosomal site of integration of the murine sarcoma virus (MSV) genome in different MSV transformed human cells, 9. to map the sarcoma specific DNA sequences of Moloney sarcoma virus and of Kirsten and Harvey sarcoma virus in normal mouse and rat cells respectively, 10. to map the structural genes for endogenous RNA tumor viruses in different mouse strains, and 11. to study the expression of malignancy in somatic cell hybrids between virus transformed and normal human cells.

125. BIOLOGICAL AND PATHOLOGICAL PROPERTIES OF DEFECTIVE SIMIAN VIRUS 40

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Biological and pathological properties of defective simian virus 40: Low multiplicity passage of SV40 in A172 cells (human glioblastoma) results in the rapid appearance of defective viral DNA. These defectives are packaged into virions since lysates of infected cells contain interference activity and once the pattern of defective viral DNAs is established they 'breed true' during successive viral passages in A172 cells. The defective viral DNAs have been mapped and contain, in addition to the viral origin, sequences for what is normally the terminus of replications. Both sequences are covalently linked and reiterated 2 or 3 times to form the defective viral DNAs. Studies have been conducted with other cell types, neural and non-neural in origin, and with other papovaviruses. Defectives are generated in human fetal brain cultures after low multiplicity infection with SV40 or BK virus and in mouse fetal brain cultures with polyoma virus. In the above cases the cells are killed fairly rapidly after infection. In LLc MK2, a Rhesus monkey kidney cell line, defectives also appear following low multiplicity infection with SV40 but the cells are killed very slowly. We are in the process of mapping these defectives. Some of the SV40 defectives generated in fetal brain cells do not contain the viral terminus but one does.

We have inoculated both wild type and defective SV40 directly into the brains of 6 Rhesus monkeys. However, after 2 years there has been no evidence of disease. We have been unable to obtain neural tissue from monkeys or humans with 'spontaneous' progressive multifocal leukoencephalopathy.

BIBLIOGRAPHIC REFERENCES: O'Neill, F.J., and Carroll, D., Appearance of defective simian virus 40 following infection of cultured human glioblastoma cells. Virology 87: 109-119, 1978. Carroll, D., and O'Neill, F.J., Genome maps of simian virus 40 defectives propagated in human glioblastoma cells. Virology 87: 120-129, 1978.

126. VIRAL TRANSFORMATION MOLECULAR BASIS - DNA VIRUS

Weissman, S. M., Yale University, School of Medicine, Dept. of Human Genetics, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

Work is continuing on the study of the possible functions of cell sequences homologous to SV40 viral sequences. In particular, we have found that the interspersed highly repetitive DNA consists of nucleoside stretches of about 265 base pairs, showing strong homology with one another, and showing some homology with the origin of replication of both the viruses. These DNA sequences act as templates in vitro for polymerase II transcription and we are currently examining the RNAs produced in vivo that are homologous to these sequences and testing whether they also serve some role in initiation and replication of DNA.

127. MECHANISMS AND REGULATION OF GENE EXPRESSION

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The major objectives of GM13235 are to use the SV40 minichromosome as: 1) A model for exploring the organization, expression and regulation of mammalian genomes. To this end we will study the ability of specific deletion mutants to express various regions of their genome as mature mRNAs. Our aim is to correlate particular segments of SV40 DNA with specific functions in transcription, processing and translation of its genetic information. 2) A vector for transducing selected genes into cultured mammalian cells, thereby permitting more controlled study of their expression and regulation in either episomal or integrated states. All or part of SV40 DNA has been or will be recombined with various bacterial, yeast, viral and mammalian genes to permit their introduction, propagation, integration and expression in established mammalian cell lines. Of particular interest is obtaining vectors with genes whose products provide a selection for cells carrying those genes.

BIBLIOGRAPHIC REFERENCES: Mutational alterations within the SV40 leader segment generate altered 16S and 19S mRNAs. Villarreal, L., White, R.T., and Berg, P. J. Virol. 29:209 (1979). Synthesis of rabbit beta globin in cultured monkey cells following infection with a SV40 beta globin recombinant genome. Mulligan, R.C., Howard, B.H., and Berg, P. Nature 277:108 (1979).

128. DNA AND RNA TUMOR VIRUSES - MOLECULAR MECHANISMS OF INFECTION AND CARCINOGENESIS

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We are studying the organization and expression of SV40 and adenovirus 2 genomes contained in Ad2-SV40 hybrid virus DNA molecules. The hybrid viruses are also used as models for understanding the behavior of integrated genomes. We are also analyzing the behavior of SV40 and Ad2-transformed or lytically infected cells as a function of the molecular conformation of viral nucleic acids within these cells, and as a function of events which may control (or be controlled by) viral replication, transcription, and translation. Secondly, we are analyzing the state and expression of the endogenous AKR murine leukemia virus

genome in mice with varying expression of leukemia and virus production, as well as exploring the evolution, transmission, and relation to leukemogenesis of various murine RNA viruses. Finally, we are concerned with production and purification of Epstein-Barr virus DNA and the use of this reagent to explore molecular mechanisms of infection and oncogenicity associated with this human herpes virus. Studies of cell lines from human lymphomas include surface markers, microviscosity, karyotypes, presence of and infectibility by EBV, growth characteristics, and morphology. New EBV strains (African and American) are studied, and we are examining the regulation of B cell activation by EBV.

BIBLIOGRAPHIC REFERENCES: Chattopadhyay, S.K., Jay, G., Lander, M.R., and Levine, A.S.: Correlation of induction of transcription of the AKR mouse genome by 5-iododeoxyuridine with the activation of an endogenous murine leukemia virus. Cancer Research 39: 1539-1546, 1979. Jay, G., Jay, F.T., Friedman, R.M., and Levine, A.S.: Biosynthesis, immunological specificity, and intracellular distribution of the simian virus 40-specific protein induced by the nondefective hybrid Ad2 ion NDl. J. Virol. 26: 411-419, 1978.

129. MOLECULAR BASIS OF GENETIC RECOMBINATION (BACTERIA, VIRUSES)

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The summary for this project is under entry no. 65.

130. STRUCTURAL BASIS FOR REGULATION OF DNA VIRUS GENE EXPRESSION

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The summary for this project is under entry no. 99.

131. MOLECULAR BIOLOGY OF SV40 MUTANTS

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This is part of a broader project. A summary of this subproject is not available.

2. VIRUS REPLICATION

132. INTEGRATION AND TRANSCRIPTION OF THE SV40 GENOME IN TRANSFORMED HUMAN-MOUSE CELL HYBRIDS

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OBJECTIVE: The analysis of the integration and transcription of the viral DNA in transformed cells, with the aim of finding a possible relationship between the two patterns.

APPROACH: Building of the integration map: DNA from transformed mouse-human hybrid cells is restricted with a variety of restriction enzymes and hybridized to radioactive viral DNA, following the Southern transfer technique. Building of the transcriptional map: radioactive RNA from the same cells is hybridized to restriction fragments of viral DNA immobilized on nitrocellulose and, conversely, radioactive viral DNA is hybridized to RNA immobilized on DMP-paper.

PROGRESS: The two clones of cell hybrids contain tandemly linked integrated SV40 genomes, some of which are deleted. One clone contains three times as much viral DNA (Cell (1978), 15, 1411-1426). This clone produces three times as much viral RNA and T-antigen (in progress).

133. THE PROCESS AND CONTROL OF TRANSCRIPTION OF SV40

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Our primary objective is to gain a thorough understanding of the relation of simian virus 40 (SV40) chromatin structure to its function as a template for transcription and replication. We have previously shown that the intracellular SV40 chromatin is heterogeneous with respect to its ability to function as a template, with recently replicated ('new') viral DNA serving preferentially. We are examining the possibility that an interaction with capsid protein(s) is responsible for the diminished template activity. A comparison of the structures of 'new' and 'old' viral chromatin is being made with the use of various nucleases.

We have recently shown that histone-deficient SV40 chromatin cannot replicate without the prior and/or concomitant addition of histones. Further studies will be aimed at determining the basis for the requirement of histones in the process of viral replication.

134. ANALYSIS OF EARLY SV40 TRANSCRIPTION

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Early deletion mutants of SV40 were studied in an attempt to determine those nucleotide sequences which affect the processing and stability of the two major early SV40 mRNAs. A mutant with a deletion located entirely within the coding region for large T-antigen generated a stable shortened large T-mRNA. In contrast, a deletion removing both large T and small t splice junctions as well as intervening sequences produced no detectable stable early mRNA.

The early SV40 mutants with deletions between 0.53 and 0.60 map units (54/59 mutants) are viable in tissue culture infections. While all of these early viable mutants contain deletions in the intervening sequences for the large T-antigen, results from transcriptional mapping showed that none of these deletions appear to affect the sites or frequency of splicing for large T-mRNA. The 54/59 mutants in which the deletion removes the proximal small t splice junction make no discrete stable cytoplasmic small t-mRNA. Those 54/59 mutants with deletions that do not include the splice junction synthesize discrete and predictably shortened small t-mRNAs. The size and location of the specific deletions, however, appear to significantly affect the abundance of the altered transcripts, this probably reflects the frequency with which the splice is made. These findings suggest that both the primary sequence near the splice junctions and the secondary or tertiary RNA structure influence the location and frequency with which a particular splicing event occurs.

In addition, SDS-polyacrylamide gel analysis of the early SV40 polypeptides synthesized by the 54/59 mutants has suggested a direct relationship between the abundance of an mRNA and the amount of the corresponding polypeptide. These results suggest that the synthesis of the early SV40 proteins is regulated primarily by the synthesis and processing of a particular mRNA, rather than by translational or post-translational controls.

BIBLIOGRAPHIC REFERENCES: Khoury, G., Gruss, P., Dhar, R., and Lai, C.-J.: Processing and expression of SV40 mRNA: The effect of early deletions. Cell, 1979, in press.

135. REGULATION OF IN VITRO TRANSCRIPTION OF SV40 BY T-ANTIGEN

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The SV40 gene A product, large T-antigen, is a protein of 96,000 daltons which is expressed early after infection of host cells. Large T-antigen is required for the establishment of the transformed phenotype in nonpermissive hosts and plays a regulatory role in initiating viral DNA replication and transcription in permissive hosts. Experiments in vivo with temperature-sensitive gene A mutants show that early viral RNA is overproduced at nonpermissive temperatures, indicating that T-antigen may regulate its own transcription. Recent work by my sponsor has shown that T-antigen binds selectively to three sites on SV40 which cover the region where transcription of the SV40 early genes is thought to originate. This suggests that T-antigen may regulate RNA synthesis by interacting directly with a region of DNA adjacent to or overlapping the RNA polymerase initiation site(s).

I propose to study the transcriptional regulation of SV40 genes in vitro by using cloned DNA templates and a combination of purified RNA polymerase II and partially fractionated animal cell extracts. First, I plan to demonstrate that faithful transcription of SV40 genes can be obtained reproducibly by

this in vitro reconstituted system. This will be accomplished by using DNA templates of short and defined length so that a 'run-off' transcript originating from a putative 'promoter' site can easily be distinguished from background transcriptional products on the basis of electrophoretic mobility. Next, I intend to characterize and map the primary transcripts synthesized in vitro to confirm the identity of these discrete RNA molecules. A combination of methods including hybridization of RNA products with restriction fragments of the SV40 early genes and T1-oligonucleotide determination will be used. My major effort will then be directed towards manipulating the in vitro system so that transcription of the SV40 genes can be regulated by exogenously added purified large T-antigen. Finally, it may be possible to use cloned DNA templates containing mutant T-antigen binding sites and/or RNA polymerase initiation sites to characterize further the regulatory mechanism of transcriptional control in SV40.

136. STUDIES OF THE CONTROL OF SIMIAN VIRUS 40 TRANSCRIPTION AND PROTEIN SYNTHESIS

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We have examined the control of gene expression in simian virus 40 (SV40). There are two mRNAs transcribed from the early region both of which arise from a common precursor. The relative proportion of the RNAs is not influenced by a mutation in large T-antigen, the SV40 specific protein which controls the transcription of these RNAs. However, the temperature of growth does alter the proportions of the two RNAs. This apparently occurs by shifting the splicing of the precursor RNA in favor of one of the RNAs. We believe that this shift indicates a type of control of gene expression which involves the stability of structures formed in the RNA template which are required for splicing. With changes in metabolic environment (i.e., temperature, pH, or ionic strength) the stability of a splicing structure would change; thus a precursor RNA, able to be spliced in several ways, may be shifted toward splicing of one RNA which codes for a specific protein needed by the cell (or virus) under a particular growth condition. Experiments to confirm this model of gene expression are in progress.

In other experiments, we have studied the control of late transcription of SV40. These studies indicate a continual requirement of the SV40 early protein, large T-antigen, for late transcription. However, a host cell factor is probably involved as well and the interaction of this factor and large T-antigen varies between cell lines. Also studied is the synthesis of SV40 RNA in transformed cells.

BIBLIOGRAPHIC REFERENCES: Adams, S.L., Alwine, J.C., deCrombugghe, B. and Pastan, I. Identification of two collagen RNAs larger than Pro-α1 and Pro-α2 collagen mRNA which are decreased in transformed fibroblasts. J. Biol. Chem., 1979, in press.

137. TRANSCRIPTION OF SV40 DNA-PROTEIN COMPLEXES BY DNA-DEPENDENT RNA POLYMERASE II FROM BSC-1 CELLS

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For the past few years, we have been interested in establishing conditions for the study of the components necessary for correct in vitro synthesis of specific RNA molecules. We have established a procedure for the isolation of DNA dependent RNA polymerase from nuclei of BSC-1 cells in culture. These cells are permissive to infection by the transforming virus SV40. We have begun experiments to analyze the transcription of SV40 DNA-protein complexes by relatively crude preparations of RNA polymerase isolated from cells infected with SV40. We have found that under certain conditions, we can achieve asymmetric transcription of the SV40 DNA-protein complexes such that the correct strand from both the early and late regions of the virus are used by the enzyme. Current studies are directed towards determining components necessary for proper strand selection.

138. CONSTRUCTION AND CHARACTERIZATION OF DELETION MUTANTS OF SIMIAN VIRUS 40 LACKING PRESUMED REGULATORY DNA SEGMENTS

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The aim of the proposed project is to understand the role of the intervening sequences in the transcription of simian virus 40 late mRNAs, and the function of SV40 16S leader RNA in transcription and translation.

The experimental approach will be to construct in vitro deletion mutants of SV40 lacking either one of the DNA sequences in question or both of them, and then to characterize the alterations in transcription or translation of the mutants' late mRNAs.

Through the correlation of the absence of a particular DNA segment with a specific biological defect, I hope to clarify the role of these presumed regulatory regions.

139. GENE STRUCTURE IN ONCOGENIC VIRUSES

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We propose to characterize the gene characterization of SV40 mRNA with regard to location of internal modified bases and identification of 5' terminal capped ends of minor species of mRNA. We will study the subcellular distribution of mRNA species and the type of nucleoprotein complexes in which SV40 mRNA is found. We will attempt to identify the sites of initiation of transcription for minor and major species of SV40 mRNA in vivo and in vitro and study the effect of deletion mutants on the initiation of transcription. Methods of analysis will include analysis of mRNA labelled in vivo and of cDNA extension products of mRNA and use of permeabilized cells and soluble extracts for transcription of viral DNA.

140. PURIFICATION AND INVESTIGATION OF THE BIOLOGICAL PROPERTIES OF SV40 SMALL-T MRNA

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To investigate the function of individual SV40 tumor antigens, we have purified the mRNAs for SV40 small-t and large-T and are attempting to introduce the purified mRNAs into living cells. In this way it may be possible to observe the biological effects of the SV40 proteins following their synthesis in injected mammalian cells. For the first experiments, however, we have used purified small-t mRNA and injected it into Xenopus oocytes.

A fragment of SV40 DNA containing the sequences between 0.59 and 0.54 map units (which encode the unique portion of SV40 small-t) was isolated using the restriction enzymes Hae III and Alu I. The purified fragment was cloned in the plasmid vector pBR322 (under GMAG containment category 2). The linearized plasmid DNA was covalently attached to cellulose and the resin used to isolate small-t mRNA by preparative scale hybridization.

The purity of the mRNA was checked by translation in a cell-free system. This showed that the purified fraction contained mRNA highly active in the synthesis of small-t with virtually no contamination with mRNA coding for SV40 large-T.

To test the biological activity of the purified mRNA it was injected into Xenopus oocytes. Pure small-t mRNA directed the synthesis of small-t but not of large-T, whereas total SV40 mRNA directed the synthesis of both large-T and small-t. This result suggests that small-t mRNA is not spliced to give large-T mRNA in the cytoplasm of living cells.

Further experiments in which we inject mRNA into the oocyte nucleus and into mammalian cells are in progress.

141. CONTROL OF GENE EXPRESSION IN TUMOR VIRUSES AND CELLS

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The overall objective of this proposal is to systematically develop our understanding of gene regulation in virus-infected

cells and to use this knowledge for developing in vitro systems from which activities can be purified and characterized. As this is a renewal of research in progress, all the outlined projects have already produced promising results. The following is a list of these projects which deal with four viruses: the papovaviruses SV40 and polyoma, the parvovirus MVM and the retrovirus MLV. Studies with SV40 and polyoma: 1) Purification, characterization and biological functions of poly(A)-negative viral-specific RNAs. 2) Purification, characterization and biological functions of small viral RNAs. 3) Purification, characterization and biological function of high molecular weight SV40 RNA. 4) 'Attenuation' as a mechanism for regulating transcription. 5) Isolation, purification and characterization of various forms of viral minichromosomes. 6) Characterization of minichromosomes of substituted SV40 DNA molecules. 7) Characterization of minichromosomes having transcriptional activities. 8) Transcriptional initiation in vitro using either E. coli polymerase or eukaryotic polymerase II and viral minichromosomes. 9) The relation of the cytoskeleton structure to mRNA biogenesis and cellular architectural features that affect genomic activity. 10) Analysis of polyoma nuclear RNA. Studies with MVM: 1) Characterization of early and late viral RNAs. 2) Transcriptional initiation and capping of MVM RNAs. 3) Isolation and characterization of transcriptional complexes. 4) Splicing as a mechanism regulating permissivity. 5) Isolation, characterization and biological functions of 'lollipop' and circular MVM DNA structures. 6) The possibility that MVM DNA has a minichromosome structure. Studies with MLV: Characterization of poly(A)-positive and poly(A)-negative viral RNAs.

In addition, the viral RNAs of the various systems will be used as substrates in in vitro studies aimed at isolation and characterization of splicing activity. The various RNAs that will be used are: a) Nuclear and cytoplasmic 19S SV40 RNAs; b) nuclear MVM RNA; and c) MLV genomic 35S RNA.

142. REGULATION OF EXPRESSION OF THE GENOME OF SIMIAN VIRUS 40

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The complete nucleotide sequence of simian virus 40 DNA is now available. Fourteen percent of the DNA does not code for protein. A good part of this sector consists of regulatory sequences such as those involved in initiation of transcription and post-transcriptional processing of viral RNA molecules. The objective of this proposal is to correlate nucleotide sequence of the viral DNA with its function in regulation of the DNA involved in regulation of genome expression. Our approach is to construct viral deletion mutants lacking one specific sequence at a time and to examine the effect on genome expression. Sequences to be examined in this way are: a) The 203 nucleotide long leader sequence of the major late viral messenger RNA (we have already constructed mutants lacking this sequence using a procedure developed by me and mapped the deletions accurately by DNA sequencing); b) sequences involved in 'splicing' of the late viral mRNAs; c) three sets of reiterated sequences occurring in the beginning of the late region; and d) sequences involved in transcription-termination, or processing at the 3' ends of viral RNAs. Emphasis will be placed on studying the pattern of RNA synthesis in mutants lacking each of these sequences to determine whether transcription or processing of the viral RNAs is affected, and, if processing if affected, the nature of unprocessed (or slowly processed) intermediates that accumulate.

143. ASPECTS OF CONTROL IN MAMMALIAN GENE EXPRESSION

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The basis of gene amplification in PALA-resistant hamster cells which overproduce carbamyl-P synthetase, aspartate transcarbamylase and dihydro-orotase (as a multifunctional protein) and the corresponding mRNA will be investigated, using cloned cDNAs derived from the purified mRNA and cloned restriction fragments of genomic DNA. The structures of the mRNA and genomic DNA will be compared, and we will perform experiments with cells in which we will attempt to alter the frequency with which PALAr mutations arise. We will use specific antibodies to the SV40 proteins VP3 and T antigen to investigate further their in vivo functions by determining their locations on SV40 chromatin, and also determining the nature and role of post-translational modification of these proteins. We will explore the function of pppA2'p5'A2'p5'A as an effector for a nuclease concerned with regulation of mRNA levels in mammalian cells, and try to elucidate the details of this regulatory mechanism.

BIBLIOGRAPHIC REFERENCES: Wahl, G.M., Stern, M. and Stark, G.R. (1979) Efficient Transfer of Large DNA Fragments from Agarose Gels to Diazobenzyloxymethyl-Paper and Rapid Hybridization Using Dextran Sulfate, Proc. Natl. Acad. Sci. USA 76, 3683-3687. Reiser, J., Renart, J., Crawford, L.V. and Stark, G.R. (1980) Specific Association of Simian 40 Tumor Antigen with Simian Virus 40 Chromatin, J. Virology, in press.

144. GENE REGULATION IN MAMMALIAN CELLS

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The objective of this research proposal is to use SV40 as a simple model to study the basic mechanism of gene regulation of mammalian cells. We plan to: 1. Analyze the mechanism for the production of SV40 mRNA by studying the relationship between various post-transcriptional processing steps for the generation of mature mRNA. 2. Analyze the regulation of SV40 late gene expression during early or abortive infection by analyzing the rate of late mRNA synthesis and its subsequent processing. 3. Analyze the secondary structure of SV40 late nuclear RNA cross-linked in vivo in the search for structural basis of splicing.

145. REGULATION OF REVERSION IN SV40 TRANSFORMED RAT CELLS

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We are currently studying the mechanisms of growth control in a normal rat embryo fibroblast line, an SV40 transformed tumorigenic line produced from these cells, and a set of T antigen-negative revertants isolated from the transformed line. Special emphasis is being placed on the mechanism of regulation of the integrated viral genes in that revertant which still contains the viral genome. The procedures we will use include the transfection of cellular DNA and microinjection of DNA and/or RNA into cells. It is hoped that we will be able to determine whether the reversion was caused by a change in the early region of the virus, rendering it nonfunctional, or by an active cellular suppression of the expression of the integrated genome.

It is our hope that these studies will provide some insight into the ways in which the normal growth controls of a cell can be perturbed by an external agent such as a chemical carcinogen, a virus, or radiation, resulting in a cancer cell.

BIBLIOGRAPHIC REFERENCES: Pollack, R., B. Steinberg, S. Zucker, W. Topp and M. Botchan. Negative selection for revertants from transformed rat cells carrying one copy of SV40 DNA yields T-antigen-negative clones, in Early proteins of oncogenic DNA Viruses, INSERM, Paris, pp. 311-316 (1977). Steinberg, B.M., Pollack, R., Topp, R., and Botchan, M., Isolation and characterization of T-antigen-negative revertants from a line of transformed rat cells containing one copy of the SV40 genome. Cell 13: 19-32 (1978).

146. CONTROL OF TUMOR VIRUS GENE EXPRESSION

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The major purpose of this study is to explore control of SV40 gene expression, both in lytically infected and in virus-transformed cells. This involves the location of regions of the SV40 genome involved in control of gene expression, the isolation of sets of mutants with lesions in these regions, and the use of these mutants to probe control of viral gene expression. We are currently engaged in the following specific studies: 1) Detailed characterization of six new mutants with deletions between 0.199 and 0.22, all of which are viable for growth in

African green monkey kidney cell lines. We are studying their macromolecular synthesis, DNA sequence, transformation properties, and adenovirus helper function. 2) Application of recombinant DNA technology for the isolation of mutants of SV40 DNA in pure form for use in preparing mutants via D-loops and other methods and the use of recombinant DNA technology for the direct isolation of mutants of SV40. 3) Studies are under way to examine the utility of directed single-base mutagenesis in SV40. Fragments of DNA, carrying a single base alteration, are synthesized enzymatically with polynucleotide phosphorylase, annealed to single-stranded circles of SV40 DNA, and elongated enzymatically. From these heteroduplexes, mutants can be isolated. This is being done in collaboration with Dr. Michael Smith, University of British Columbia.

147. EXPRESSION OF A EUKARYOTIC GENE WITHIN AN SV40 MOLECULE

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We hope to be able to develop an efficient vector for the cloning of eukaryotic genes or structurally important fragments of eukaryotic genes in enkaryotic cells using the DNA tumor virus, SV40. Using this vector we hope to analyze in great detail the transcriptional and processing controls of a particular gene, the major mouse beta globin gene, and, in particular, to study: 1. The genetic activity of the globin sequences within the SV40 vector; in particular, which promoter and processing signals are controlling the expression of the globin gene in the various hybrid molecules constructed. 2. The effect of changes in the primary sequence in the vicinity of the splice joint on the fidelity of the splicing mechanism and perhaps gain an insight into the nature of the splicing mechanism. 3. The mechanism of the increase in globin synthesis in induced mouse erythroleukemia cells using very specific beta globin probes amplified via the SV40 vector.

148. ROLE OF SPLICING IN GENE EXPRESSION

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Small DNA tumor viruses, like SV40, provide an excellent model system for studying regulation of eucaryotic transcription. We have been interested in the role of RNA splicing in the biogenesis of SV40 mRNA processing. Mapping studies indicate that the spliced cytoplasmic viral mRNAs are derived from the colinear nuclear transcripts which contain intervening sequences (IVS). If the IVS have acquired some essential functions, deletion should lead to a defect of this function. Thus, in an attempt to determine the function of the IVS we have generated a deletion mutant lacking precisely the IVS of 16S mRNA. Although this mutant contains the entire information of VP-1, it was unable to synthesize any stable 16S mRNA and consequently no VP-1. The nature of this failure is presumably due to a defect in the post-transcriptional processing of the viral RNA. This presumption is supported by studies with another set of deletion mutants located at or near the splice junction which decrease the frequency with which the splicing event occurs. These results indicate that splicing is an essential function in the biogenesis of SV40 mRNAs.

BIBLIOGRAPHIC REFERENCES: Lai, C.-J., Dhar, R., and Khoury, G.: Mapping the spliced and unspliced late lytic SV40 RNAs. Cell 14: 971-982, 1978. Gruss, P., Lai, C-J., Dhar, R. and Khoury, G.: Splicing as a requirement for biogenesis of functional SV40 mRNA. PNAS, 1979, in press.

149. DIFFERENTIATION OF MURINE TERATOCARCINOMA CELLS PERMITS SV40 GENE EXPRESSION

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Undifferentiated murine teratocarcinoma cells do not support SV40 or polyoma virus replication nor the expression of the early papovavirus proteins. The F-9 line of murine teratocarcinoma is particularly suited for induction studies since it undergoes very limited differentiation under normal growth conditions. We have shown in earlier studies that undifferentiated F-9 cells, infected with SV40 at high multiplicities, synthesize virus-specific RNA representing a full but non-spliced early transcript. We now show that differentiation of the cells in vitro using retinoic acid induces early SV40 gene expression in the differentiated cells.

BIBLIOGRAPHIC REFERENCES: Segal, S., Levine, A.J. and Khoury, G.: Evidence for non-spliced SV40 mRNA in undifferentiated murine teratocarcinoma cells. Nature, 1979, in press.

150. FUNCTION AND EXPRESSION OF SV40 VIRAL TUMOR ANTIGENS

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SV40 T antigen is involved in malignant cell transformation and viral replication. According to biological and biochemical evidence this viral gene product accomplishes its potent effects via its interactions with cellular and viral DNA. The properties of T antigen will be examined by analysis of its post-translational modifications including phosphorylation and glycosylation. The relationship of these processes to the specific DNA affinities and oligomerization of T antigen in permissive and transformed cells will be investigated. We will attempt to isolate cellular DNA sequences in transformed cells for which active T antigen has specific affinity.

Various aspects of T antigen expression will be analyzed. mRNA known to code for the T antigens will be used to study processes such as splicing and other post-transcriptional modifications. We will investigate differences in T antigen mRNA isolated from lytically infected and transformed cells in terms of these modifications and also with regard to intracellular localization and cytoskeleton. Host regulation of T antigen mRNA expression will also be studied in revertants isolated from SV40 transformants.

151. THE EFFECT OF DELETIONS AT THE 3' END OF THE SV40 EARLY REGION ON THE ADENOVIRUS HELPER FUNCTION

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Infection of monkey cells with human adenoviruses produces a much decreased yield of virus, as compared with human cells. Co-infection of these monkey cells with SV40 increases the yield of adenovirus by one thousand fold. Two SV40 deletion mutants which map the C-terminus of large-T antigen, dl 1263 and dl 1265 have helper activity reduced to 30 percent and 4 percent, respectively, of that of the wild type virus at the same multiplicity of infection. Both deletions are multiples of three nucleotide pairs and do not therefore affect the reading frame used in translation of large-T (Van Heuverswyn et al., Journal of Virology, 1979, 30, 936). The properties of these mutants show the importance of the C-terminal region of large-T antigen in mediating the adenovirus helper effect of SV40.*

152. BIOLOGICAL PROPERTIES OF SV40 EARLY PROTEINS

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The goal of this project is to elucidate and characterize biological properties of SV40 early proteins. The antigenic relatedness of SV40 large T, small t, U, and S antigens was established using antiserum directed against purified, denatured T polypeptide. The presence of large T-ag in the plasma membrane of SV40-infected and transformed cells was established. Transport-defective mutants of SV40-adenovirus 7 hybrid virus (PARA) were found to exhibit a dominant effect which inhibited nuclear migration of wild-type SV40 T-ag.

153. BASIC MECHANISMS IN VIRAL CARCINOGENESIS

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Our past and future experiments are directed at: 1. Understanding the organization, expression and regulation of SV40

early genes and how their gene products function in the initiation and maintenance of the oncogenic phenotype. 2. Developing and using SV40 DNA vectors to introduce new genes into the genome of cultured cells to provide experimental models for studying the mechanisms of gene expression and regulation in mammalian cells.

Our more immediate goals towards these objectives are: 1. To construct appropriate recombinant viral genomes which can be maintained in either an integrated or autonomously replicating state in cultured animal and human cells. 2. To use such recombinant DNAs to explore the function of viral regulatory elements, particularly those that control the expression of the oncogenetic functions. 3. To analyze the patterns and mechanisms of homologous and non-homologous recombination in mammalian cells.

BIBLIOGRAPHIC REFERENCES: Synthesis of Rabbit beta-globin in Cultured Monkey Kidney Cells Following Infection with a SV40 beta-globin Recombinant Genome. R. C. Mulligan, B. H. Howard and P. Berg. Nature 277 p. 108-114 (1979). Nucleotide Sequence Analysis of Two Simian Virus 40 Mutants with Deletions in the Late Region of the Genome. R. Contreras, C. Cole, Paul Berg, and Walter Fiers. J. of Virol. 29: 789-793 (1979).

154. STUDIES ON ROUS SARCOMA VIRUS AND SIMIAN VIRUS 40

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In studies of SV40 early antigens, Ad2(plus) ND2 induced in infected cells two SV40 specific polypeptides of 42,000 and 56,000 molecular weight; Ad2(plus) ND1 induced a 28,000 molecular weight peptide. The Ad2(plus) ND1 segment is found to be sufficient to induce the SV40-specific tumor antigen in mice.

With 2-dimensional gel electrophoresis a 4th AAV structural protein (VP) was detected. Each of the four VP's (m.w. 86.5, 72.5 61.5, 60.5) contains multiple isoelectric species. While VP 1, 2 and 3 share many tryptic peptides, at least, VP 1 and 3 each contain unique tryptic peptides. Virion proteins as well as cell free translated proteins are presently being analyzed to test whether the VP's are overlapping or whether that frame shift also plays a part apparently as a method of conserving DNA by this virus.

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155. BIOSYNTHESIS IN NORMAL AND VIRUS-TRANSFORMED CELLS

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This research program is aimed at the understanding of the interaction of viral and cellular functions which determine the altered phenotype of cells transformed by DNA tumor viruses, and some of the parameters which differentiate transformed from normal cells. The project consists of 5 parts, which are listed below: 1) The study of the regulation of viral functions in SV40 or polyoma transformed cells, particularly of the integration and transcription of the viral DNA. 2) The study of the SV40 specific membrane antigens and their relationship to nuclear T-antigen(s). 3) The study of the early events in SV40 infection, with particular regard to integration. 4) The study of the cellular functions required for herpes virus DNA replication and integration. This project will take advantage of the use of temperature sensitive (ts) cell mutants with specific growth defects. 5) The study of the role of RNA unwinding protein(s) in m-RNP binding to ribosomes and m-RNA transport through the nuclear membrane in eukaryotes.

BIBLIOGRAPHIC REFERENCES: Zouzias, D., and Basilico, C. T-antigen expression in proliferating and non-proliferating SV40 transformed mouse cells. J. Virol. 30, 711-719 (1979). Basilico, C., Gattoni, S., Zouzias, D., and Della Valle, G. Loss of integrated viral DNA sequences in polyoma transformed cells is associated with an active viral A function. Cell 17, 645-659 (1979).

156. USE OF XENOPUS OOCYTES AND EGGS FOR IDENTIFICATION OF FUNCTIONAL COMPONENTS OF T ANTIGEN IN SV40 GENE EXPRESSION

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The objectives of the proposed research are to identify separate biological functions of T antigens by injection of Xenopus oocytes and eggs with DNA from SV40 early gene deletion mutants. In addition, we hope to localize an early gene deletion on the SV40 genome responsible for certain biological properties associated with T antigens. The specific aims of this proposal are: 1) To identify separate functional properties of T antigen by injecting purified DNA from various SV40 early gene deletion mutants into frog eggs or oocytes from the species of Xenopus laevis. a) To compare the ability of various early gene deletion mutants of SV40 to initiate viral specific replication upon injection. b) To compare the ability of early gene deletion mutants to transcribe SV40 late mRNA upon injection. c) To compare amounts of early mRNA synthesis between early gene deletion mutants and wild type SV40 DNA upon injection. 2) To examine biological activities of T antigens, when T antigens and DNA from SV40 early gene deletion mutants are injected into Xenopus oocytes and eggs. 3) To map on the SV40 genome, the deletion responsible for a certain early gene function. 4) To examine which restriction endonuclease DNA fragments of SV40 DNA contain the promoter sequence for early gene transcription.

157. STRUCTURAL ANALYSIS OF THE SV40 TUMOR ANTIGEN

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The purpose of the proposed research is to compare structurally several proteins observed in immunoprecipitates of SV40 infected cells. The proteins include two known products of the SV40 early genes, proteins with molecular weights of 94,000 and 17,000 daltons. Two additional proteins with molecular weights of 56,000 and 32,000 daltons are also present in cells infected with wild-type virus, but are absent from immunoprecipitates of cells infected with certain non-transforming deletion mutants. The possibility that the 56K and 32K proteins are only observed when complexed to the 17K viral gene product is being explored.

BIBLIOGRAPHIC REFERENCES: Crawford, L., C.N. Cole, A.E. Smith, E. Paucha, P. Tegtmeyer, K. Rundell and P. Berg. 1978. The Organization and Expression of Simian Virus 40's Early Genes. Proc. Natl. Acad. Sci., U.S.A. 75: 117. Rundell, K. and P. Tegtmeyer. Human Papovavirus T Antigens: Identification and Comparison with the Simian Virus 40 Protein A. In: Microbiology - 1978. D. Schlessinger,, ed. American Society for Microbiology, Washington, D.C.

158. SV40 T ANTIGEN BINDING SITES IN CELLULAR DNA

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The SV40 large T antigen is a DNA binding phosphoprotein. It may also be closely associated with a protein kinase activity and as such has the capacity to phosphorylate itself. The function of this antigen is necessary for SV40 induced neoplastic transformation, the initiation of SV40 DNA replication, and the control of early viral transcription. Large T purified from SV80 cells, an SV40-transformed line, binds preferentially to certain sites on the SV40 genome. I would first like to investigate whether the state of T phosphorylation affects these DNA binding properties. Specifically, I should like to determine whether the state of large T antigen phosphorylation affects both its quantitative ability to bind to various DNAs as meas-

ured by the nitrocellulose filter binding technique and also whether binding to certain specific sites on the SV40 genome is detected by nuclease protection experiments.

Secondly, there is immunofluorescent banding evidence which suggests that T antigen associates with mammalian chromosomes in a site specific manner. To pursue this possibility further, I wish to ascertain, directly, whether preferential binding sites for SV40 large T antigen exist in cellular DNA and, if so, to determine the nucleotide sequences of one or more of these loci. To answer these questions, I shall use a cloned 'library' of shotgun mouse cellular DNA as well as certain well-characterized cloned mouse genes. Using various techniques involving protection of selected sites from digestion by a specific nuclease, I shall attempt to identify and characterize any specific T antigen binding sequences which may exist.

159. STUDIES ON THE SV40 T-ANTIGEN BINDING SITE

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The central aim of this research is to decipher how T-antigen binds specifically to three sites on the SV40 chromosome. The approach involves the following: (1) Chemical and enzymatic synthesis of the tight-binding T-antigen site (site I). (2) Site specific insertion of purine and pyrimidine analogs into this binding site. (3) Kinetic measurements of the stability of T-antigen bound to this DNA (modified and unmodified). The approach should reveal functional groups on base pairs that are recognized by T-antigen. These syntheses and binding studies will then be extended to include sites II and III and the DNA regions containing the replication origin and the early mRNA promoter. These results will be useful for deciphering the biological mechanisms associated with SV40 replication, early mRNA transcription, and maintenance of the transformed state.

160. CENTRIOLAR ANTIGENS AND INVOLVEMENT IN CELL PROLIFERATION

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We have found that infection of TC7 cells, African green monkey kidney cells, with simian virus 40 stimulates host antigens which locate in the centriolar region of cells. The centriolar antigens consist of two polypeptides with molecular weight of about 14K and 17K daltons. The stimulation of the antigens is a result of de novo protein synthesis which occurs very early in infection, 0 to 5 hours after infection. The centriolar antigens are induced in cells infected with temperature-sensitive mutants of all complementation groups (tsA, tsB, tsC, tsBC and tsD) at restrictive temperature but not in cells infected with viable deletion mutants which contain deletions in the region of small-t gene (0.54-0.59 map units) and exhibit reduced transforming ability. Antigenically related protein(s) exists in ciliated epithelial cells isolated from chicken trachea and the related antigen(s) is localized at basal bodies of the cells. Our specific aims are: (1) To study the nature of the antigens; (2) to study the nature of the antigen stimulation; (3) to study the involvement of the antigens in various transformed cells and in cells at the particular phase of cell cycle; (4) to study the presence of the related antigens in other cells; and (5) to study the localization of antigens at the supramolecular level.

161. MECHANISM OF THE MITOGENIC ACTIVITY OF SV40 AND POLYOMA VIRUS TUMOR ANTIGENS

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OBJECTIVE: The goal of our investigations is to define the cellular processes which are activated by tumor antigens of small DNA replication and mitosis in lytically or abortively infected cells. Specifically, we are interested in those reactions which are switched on early after infection at times when the production of the virus-coded tumor antigens starts (which is probably earlier than the time when tumor antigens can be detected in infected cells by immunofluorescence). In a second project we are trying, using in vitro DNA replication systems, to trace those proteins which are necessary for the initiation of DNA replication itself, in hope of being able to define the requirements for the synthesis of these proteins.

APPROACH: We use primary mouse kidney cells which are in a resting state prior to infection. Infection of these cells with polyoma virus leads to a lytic response with an eventual destruction of cells. Prior to this, cellular DNA synthesis is initiated at about the same time as viral DNA is being replicated. Cells start an abortive mitosis which is not completed because of the lytic effect of the virus. Infection with SV40 also causes induction of cellular DNA synthesis, there is no virus DNA replication and most cells go through one division cycle. In both systems effects of virus infections are studied up to the time of the initiation of DNA replication. Chromatin structure, transcription and translation are investigated at different times after infection in order to eventually define the cellular changes induced by tumor antigens.

162. CELLULAR DNA REPLICATION IN NORMAL AND SV40 INFECTED CELLS

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OBJECTIVE: We propose to study cellular DNA replication in synchronized mammalian tissue culture cells, in cells productively infected with simian virus 40 and, eventually, in cells abortively infected with SV40 (where infection may lead to transformation).

APPROACH: In each system, fiber autoradiography will be used to determine frequency of initiation events, rate of replication fork progression, size of replication units, and whether units operate synchronously as clusters. In vitro DNA replication in a subcellular system will also be examined. We will determine how closely this mimics in vivo replication by using fiber autoradiography. Cellular DNA replication in vitro will also be examined at various points in S phase in normal and SV40 infected cells. The data should permit us to determine the contributions of these measures to overall DNA synthesis at any time during S phase, and determine whether the induced round of cellular DNA synthesis that occurs in productive and abortive SV40 infection is abnormal.

163. STUDIES ON DNA REPLICATION AND RECOMBINATION

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Nucleic acid biochemistry: We are currently involved in three lines of experimentation that deal with the biology and chemistry of nucleic acids. 1. The first line of experimentation deals with the isolation and characterization of the regulatory DNA elements which serve as the origins for DNA synthesis in the viruses phi X and G4.

By a combination of biophysical and electron microscopic techniques we can locate the regulatory sequences at which the synthesis of positive strand DNA is initiated to form rolling circle intermediates, and the point at which negative strand DNA is initiated to convert single-stranded circles to duplex rings. The latter initiation event represents the reaction leading to the formation of an Okazaki fragment. 2. A second line of experimentation involves the isolation and sequencing of the three DNA regions in bacteriophage phi-X which function as sites for the initiation of messenger RNA synthesis. 3. Our most extensive current effort involves the study of DNA recombination. We have been able to use the electron microscope to observe individual DNA molecules and analyze intermediates in the process of recombination.

Current recombination experiments under way include a) the observation of recombination intermediates in a eukaryotic system, specifically SV40 infected cells, b) the analysis of a mutation (the chi mutation) that creates hot spots for genetic recombination, and c) the development of an in vitro system for studying the enzymology of generalized recombination.

164. DNA REPLICATION IN SV40 INFECTED CELLS

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SV40 nucleoprotein complexes have been extracted from virus infected cells to follow the replication and maturation of viral DNA. Four principle forms of SV40-protein complexes

have been detected: (1) A heterogeneous collection (75S-200S) of replicating DNA molecules, (2) a 75S nuclear protein complex that is the immediate product of replicated viral DNA, (3) a 200S previrion that is derived from the 75S complex and (4) a 250S virion derived from the previrion complex. Pulse-chase and kinetic labeling experiments have been used to establish the precursor product relationship between these various forms of SV40-protein complexes. Structural studies of the nucleoprotein complexes and the proteins they contain are under way.

165. ROLE OF DNA RELAXING ENZYME IN SV40 DNA REPLICATION

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The proposed research seeks to determine the involvement of the DNA relaxing enzyme in simian virus 40 (SV40) and eukaryotic DNA replication. SV40 DNA replication can serve as a model for cellular DNA replication since, like cellular DNA, SV40 is replicated in the nucleus by cellular enzymes and is associated with cellular histones to form the characteristic nucleosome structure.

SV40 chromosomes isolated at low ionic strength from infected monkey cells retain the ability to replicate DNA in vitro when supplied with deoxy- and ribonucleoside triphosphates. Thus, at least some of the enzymes which participate in in vivo DNA replication must be bound to the replicating chromosome (RC). The DNA relaxing enzyme co-sediments with RCs (and also with non-RCs) on neutral glycerol gradients and thus may be bound to RCs and participate in their replication. Attempts to determine if the enzyme is bound by further purifying the RC have thus far been unsuccessful.

As an alternative approach, the requirement of the enzyme for in vitro SV40 DNA synthesis will be tested after addition of either a specific inhibitor (if one can be found) or anti-relaxing enzyme antibodies (if they can be raised towards the purified enzyme).

The enzyme is being purified to homogeneity from nuclei of calf liver. The physical and chemical properties of the purified enzyme will be determined.

BIBLIOGRAPHIC REFERENCES: Tsubota, Y., Waqar, M.A., Burke, J.F., Milavetz, B.I., Evans, M.J., Kowalski, D., and Huberman, J.A. (1979) Association of Enzymes with Replicating and Nonreplicating Simian Virus 40 Chromosomes, Cold Spring Harbor Symposium on Quantitative Biology, XLIII, 693-704. Kowalski, D. (1979) A Procedure for the Quantitation of Relaxed Closed Circular DNA in the Presence of Superhelical DNA: An Improved Fluorometric Assay for Nicking-Closing Enzyme, Analyt. Biochem. 93, 346-354.

166. ENZYMOLOGY OF SV40 DNA REPLICATION - IN VITRO STUDIES

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Although our current knowledge of mammalian DNA replication suggests that many enzymes and other proteins are involved, none have until recently been shown to be required. To determine which enzymes are required for DNA replication, I propose to analyze the replication in vitro of simian virus 40 (SV40) chromosomes. Replicating SV40 chromosomes can be isolated in a form which allows DNA synthesis to continue. The relative simplicity of this system should enable us to fractionate the enzymes bound to these chromosomes and determine their role(s) in DNA replication. The chromosomes will be assayed for the presence of enzymes believed to be involved in replication. For each enzyme detected, we will determine whether it is preferentially associated with replicating SV40 chromosomes. We will also examine whether the removal or inhibition of each enzyme results in an alteration in the process of DNA replication. If an alteration is detected it provides excellent evidence for the role(s) of that enzyme in the replication process; if no effect is found, the possibility of adventitious association will be tested.

BIBLIOGRAPHIC REFERENCES: Edenberg, Howard J. (1980) Extraction of transcribing SV40 chromosomes in very low salt. Nucleic Acids Research, 8, 573-586. Edenberg, Howard J. (1980) RNA- and DNA- polymerases associated with SV40 chromosomes. J. Supermolecular Structure, Supplement 4, p.334.

167. DNA POLYMERASE(S) OF THE REPLICATION SV40 CHROMOSOMES

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We intend to identify and characterize the DNA polymerase(s) which participate in the replication of simian virus 40 (SV40) DNA. Similarities between SV40 DNA replication and cellular DNA replication suggest that the same DNA polymerase(s) carry out cellular DNA replication as well. Our approach is simple: We shall purify replicating SV40 chromosomes to the maximum extent possible from infected monkey cells, then dissociate DNA polymerase(s) from the replicating chromosomes, and then use standard techniques to compare the DNA polymerase(s) so obtained with DNA polymerases alpha, beta, and gamma prepared by conventional procedures from monkey cells. Because initial purification of replicating chromosomes allows us to start with much purer material than in standard polymerase purification procedures, we feel it is worthwhile to try to purify the replication polymerase(s) to homogeneity. By partial and complete reconstitution experiments, we shall try to determine how the replication polymerase(s) interact with other proteins of the replication complex, with DNA, and with each other. If more than one polymerase species is associated with replicating SV40 chromosomes, we shall try to determine the function of each polymerase species during the replication process.

BIBLIOGRAPHIC REFERENCES: Robinson, M.K., Manly, K.F. and Evans, M.J. (1980) Immunosuppression by 334C Murine Leukemia Virus: Viral Specificity and the Activation of Immunosuppressive Effects During Cloned Viral Leukemogenesis, J. Immunol. 124: 1022-1027. Waqar, M.A., Evans, M.J. and Huberman, J.A. (1980) Processive DNA Synthesis by Partially Purified Replicating SV40 Chromosomes, Fed. Proc. 39: 1959, No. 1861.

168. RNA PRIMERS IN SV40 DNA REPLICATION

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The existence of RNA primers on about 30-60% of both long and short (Okazaki fragments) nascent DNA chains is now reasonably well established by data from subcellular as well as intact cell systems. Synthesized de novo at replication forks, their initiation does not depend on a unique DNA sequence signal and they are removed from nascent DNA chains before Okazaki fragments are joined.

Proposed in the project are experiments designed to extend our understanding of the role of RNA primers in the normal process of chromosome replication and to use this information to design proper assays for the enzymes and cofactors responsible. Specifically the experiments envisaged would be an attempt to answer the following questions: 1) Are RNA primers required for the initiation of Okazaki fragments during SV40 DNA replication, and, if so, how are they synthesized and excised? 2) How does DNA polymerase alpha specifically interact with native replicating chromosomes to allow the synthesis and completion of Okazaki fragments?

These questions will be posed in relation to simian virus 40(SV40) because SV40 provides us with a small, well defined, DNA genome that can continue 'normal' replication in either isolated nuclei or soluble nuclear extracts. The structure, composition and mechanism of DNA replication in these viral chromosomes is strikingly similar to that of the host. Since all of the substrates, templates and products of viral DNA replication are easily isolated and characterized, SV40 provides us with a homogeneous population of mammalian 'replicons' analagous to those found in mammalian chromosomes.

169. SIMIAN VIRUS 40, DNA REPLICATION AND TRANSFORMATION

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Experiments dealing with the requirements for expression of eukaryotic genes linked to papovaviral vectors are proposed. A large variety of plasmids carrying replicon sequences from polyoma virus, SV40, bovine papilloma virus and human papovavirus BK will be utilized. To these plasmids will be added, in some cases, the thymidine kinase gene from herpes simplex virus type I. Both morphological and biochemical transformation of normal and thymidine kinase mutant cell lines will be performed. Subsequently, the state and arrangement of plasmid-viral DNA sequences will be characterized. We anticipate that certain host cell lines will maintain specific plasmid-viral recombinants in a free replicating form which can be recovered after transformation of E. coli with nonchromosomal DNA preparations.

Studies of transcription and translation of cloned mouse immunoglobulin genes inserted into the late gene region of papovaviral vectors will be performed using lytic propagation with SV40 early mutant helpers. Finally, SV40 or BK virus genomes will be linked to transforming sequences of adenovirus types 2 and 5 of bovine papilloma virus to examine gene expression from these transforming regions and biological properties of the primate cell lines carrying such recombinants.

BIBLIOGRAPHIC REFERENCES: Purchio, A.F. and Fareed, G. C. (1979) Methods for Molecular Cloning in Eukaryotic Cells. Methods in Enzymology, R. Wu and S.N. Cohen (eds.), Academic Press, New York, in press. Purchio, A.F., Fareed, G.C. and James E. (1979) Cloning the argF Gene from Escherichia coli K-12 with Simian Virus 40. Gene 7, 15-31.

170. DNA REPLICATION IN SV40 INFECTED CELLS

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No summary has been provided to the Smithsonian Science-Information Exchange.

171. REPLICATION AND PROPERTIES OF DEFECTIVE-SUBSTITUTED SIMIAN VIRUS 40 IN PERMISSIVE MONKEY KIDNEY CELLS

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The structures of a group of newly and independently isolated defective substituted variants of SV40 were determined. These variants were initially detected by screening for variants containing monkey DNA sequences homologous to a monkey DNA segment found in a previously characterized variant. Five independent new isolates proved to be essentially identical to the variant obtained previously. The results suggest that SV40 variants do not arise totally at random but rather that certain structures recur. The close similarity between these structures suggests that some mechanism other than recombination between the monkey genome and the genome of the infecting virus may be involved in their formation.

A recombinant 'library' of monkey (C. aethiops) DNA in bacteriophage lambda has been constructed and is being used to investigate those monkey DNA segments that occur in the defective SV40 variants.

BIBLIOGRAPHIC REFERENCES: Winocour, E., Singer, M.F. and Kuff, E.: The rapid detection, isolation and amplification of host-substituted SV40 variants. Cold Spring Harbor Symposium on Quantitative Biology, Vol. 44, 1979, in press. Singer, M.F. and Thayer, R.E.: The preferential replication of a class of host-substituted defective simian virus 40 variants at low temperature. J. Virol., in press, 1980.

172. SEGREGATION OF NUCLEOSOMES DURING CHROMATIN REPLICATION

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The primary objective of the proposed research is to determine the mechanism of nucleosome segregation and its specific relationship to DNA replication. For this purpose, simian virus 40 (SV40) chromosomes will be used as a suitable but relatively simple model system.

The extent of asymmetry of nucleosomes distributed between the two arms of a DNA replication fork will be analyzed in two ways. First, newly synthesized nascent DNA in purified replicating SV40 chromosomes will be separated into nucleosomal and non-nucleosomal DNA by its sensitivity to micrococcal nuclease. The nucleosomal DNA will then be anncaled to the separated strands of SV40 DNA restriction fragments taken from either side of the origin of DNA replication in order to quantitatively assess the frequency of nucleosomes on the forward and retrograde sides of a replication fork. The extent of asymmetry will then be mapped as a function of the distance in nucleotides from the replication fork; the number of nucleotides pulse-labeled at the 3' termini will be determined by comparing the time required for exonuclease III to digest the nascent DNA chains with the time required for this enzyme to digest uniformly labeled SV40 DNA restriction fragments of known length. To determine whether pre-fork ('old') nucleosomes are distributed randomly or conservatively, experiments like the one above will be performed in the presence of cycloheximide to inhibit histone synthesis, or in the presence of unlabeled excess SV40 DNA, to prevent the association of histone octamers with newly synthesized endogenous DNA. Density labels will also be employed to separate nucleosomes composed of new (heavy) octamers from those of old (light) octamers. The second method will allow me to determine the relationship of these experiments on SV40 chromosomes with cellular chromatin. The ratio of specific exonuclease digestion in the 3' to 5' direction should directly reflect the degree of symmetry in nucleosome placement since exonucleases do not digest through mature nucleosomes on native replicating chromosomes. This assay, which does not require a restriction endonuclease map, is applicable to cellular as well as viral chromatin.

173. REPLICATION AND REPAIR OF VIRAL DNA AND RNA COMPLEXES

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The overall purpose of this project is to help elucidate the relationship between structure and function of viral and ultimately cellular nucleoprotein complexes. The interaction between cellular expression and viral genome structure will also be analyzed, in both bacterial and animal virus systems. In addition to biochemical approaches, I plan to take advantage of the permeability of cells and virus particles to psoralen derivatives such as 4,5',8-trimethylpsoralen (trioxsalen). These components cross-link the two strands of DNA in the presence of long wavelength ultraviolet light and have been shown to be useful probes for the intracellular 'nucleosome' structure of cellular chromatin and viral DNA-protein complexes. They also provide a powerful tool for an analysis of the mechanism of cellular recombination and repair. Because of both the specificity of psoralen compounds for accessible regions of DNA such as internucleosomal bridges, and the ability to study intracellular and viral structures in vivo without prior disruption of the biological system, this approach will allow a detailed evaluation of the significance of the results from in vitro studies of nucleosome structure, DNA replication, recombination and repair enzymes, and viral genome structure.

The projects currently under way can be divided according to the approach and system employed: (1) The structure of virion and intracellular SV40 nucleoprotein complexes will be analyzed in order to study the relationship between nucleoprotein structure, the function of tumor antigen, and the regulation of transcription and replication, employing both in vivo cross-linking and in vitro replication systems. (2) The intracellular structure of procaryotic 'chromatin' will be investigated with respect to its protection from photoaddition of trioxsalen derivatives. A comparison will be made between E. coli DNA, lambda prophage DNA and super-infecting lambda DNA. (3) Photochemical inactivation of several virus classes will be used as a probe for the relationship between structure and biological activity.

BIBLIOGRAPHIC REFERENCES: Doern, G.V., Siebers, K.G., Hallick, L.M. and Morse, S.A. (1980) Antimicrob. Agents and Chemotherapy 17, 24-29, Antibiotic Susceptibility of beta-Lactamase-Producing Strains of Branhamella (Neisseria) catarrhalis. Hallick, L.M., Hanson, C.V., Cacciapuoti, J.O. and Hearst, J.E. (1980) Nuc. Ac. Res. 8, 611-22, Photoaddition of trimethylpsoralen as a probe for the intracellular organization of Escherichia coli DNA.

174. TUMOR VIRUS DNA REPLICATION - A PROBE INTO ONCOGENESIS

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The objective of this research is to accurately define the sequence of molecular and enzymological events that occur during the replication of mammalian chromosomes. We are using simian virus 40 chromosomes as a suitable and relatively simple model of a single mammalian replicon. We have developed several subcellular systems that can faithfully continue both DNA replication and chromatin assembly in vitro in order to study the replication of normal endogenous viral nucleoproteins rather than purified DNA added to cell lysates and purified proteins. Our task is to fractionate these subcellular systems in an effort to isolate and characterize cellular and viral factors that are involved in the replication of mammalian chromosomes. We are now asking four specific questions. Are RNA primers required for the initiation of Okazaki fragments, and, if so, how are they synthesized and excised? How does DNA polymerase alpha specifically interact with native replicating chromosomes to allow the synthesis and completion of Okazaki fragments? What is the relationship between the initiation of Okazaki fragments and the phasing of pre-fork nucleosomes? What is the mechanism of separation of progeny DNA molecules and termination of DNA synthesis? We hope to combine the answers to these questions with data on the structure of replicating chromosomes in an effort to provide a comprehensive view of replicating chromosomes.

BIBLIOGRAPHIC REFERENCES: DePamphilis, M.L., S. Anderson, R. Bar-Shavit, E. Collins, H. Edenberg, T. Herman, B. Karas, G. Kaufmann, H. Krokan, E. Shelton, R. Su, D. Tapper, and P.M. Wassarman. The Replication and Structure of Simian Virus 40 Chromosomes, Cold Spring Harbor Symposium Quant. Biol., in press, 1979. Shelton, E.R., P.M. Wassarman, and M.L. DePamphilis. The Structure of Simian Virus 40 Chromosomes in Nuclei From Infected Monkey Cells, J. Mol. Biol. 125. 491-514 (1978).

175. SV40 T-ANTIGEN FUNCTION AND EXPRESSION

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These experiments were initiated in the Weizman Institute and are being continued at the N.I.H. Genetic and biochemical evidence obtained in several laboratories has defined the product of the SV40 A gene, the T-antigen, as a protein which is involved in viral DNA synthesis and RNA transcription. A specific interaction of this protein with the DNA sequences on the viral genome where these processes originate (termed the origin) has been established. We have undertaken an analysis of T-antigen in different forms with respect to its functional association with DNA. The data suggest that newly synthesized T-antigen may be modified in the infected cell before it will bind specifically to the origin. Either during, after, or instead of its specific interaction with DNA, the T-antigen forms oligomers which are no longer active, and which represent the majority of the T-antigen in the cell.

176. CHARACTERIZATION OF THE C-TERMINAL REGION OF SV40 T-ANTIGEN

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The large-T antigen polypeptides produced in cells infected with SV40 deletion mutants dl 1263 and dl 1265 have been characterized by analysis of their tryptic peptides with particular emphasis on the C-terminal region. The T-antigen of the wild type SV-S produces a proline-rich C-terminal peptide which is lacking in digests of dl i265 T-antigen. This defines the location of this peptide at the C-terminus of the protein and confirms the reading frame used in the translation of this part of the genome. The altered C-terminus of dl 1265 T-antigen is cysteine rich and this affects the electrophoretic properties of polypeptide markedly. The proline-rich terminal peptide is still present in digests of dl 1263 large-T antigen showing that translation continues after the deletion and terminates normally. Because of the deletion in dl 1263 the molecular weight of its large-T antigen should be slightly less than that of SV-S. In fact, its apparent molecular weight is considerably less than that of SV-S. Since dl 1263 T-antigen retains the proline-rich C-terminal tryptic peptide we can be certain that this larger than expected reduction in apparent molecular weight is not due to loss of the C-terminal region by premature termination of translation or by proteolytic degradation of the completed polypeptide.

Both dl 1263 and dl 1265 transform mouse cells and the major forms of T-antigen produced in the transformed cells were indistinguishable from those produced in infected cells. They also appeared to associate with the host 53K to form a complex in the same way as the SV-S large-T antigen (Lane and Crawford, Nature, 1979, 278, 261).

177. TEMPERATURE-SENSITIVE INDUCTION AND ASSOCIATION OF A LARGE 53K PHOSPHOPROTEIN WITH LARGE-T ON 3T3 CELLS INFECTED WITH SV40 TSA30

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Infection of 3T3 cells with wild type SV40 (a non-permissive system of infection) results in induction of a host 53K phosphoprotein that binds to large-T (see Report 71). Infection with tsA30 at the non-permissive temperature does not result in induction of this protein although large-T and small-t are expressed. The presence of the 53K protein was tested using anti-53K serum. 3T3 cells transformed by ts-A30 express the 53K protein when maintained at the permissive temperature. Pulse chase experiments indicate that the 53K-large-T complex in these cells dissociates more rapidily at the non-permissive temperature (t1/2,4h) than the permissive temperature (t1/2, 24h). Most of the large-T synthesized following a shift-up to the non-permissive temperature fails to associate with the cellular 53K protein, although normal amounts of 53K are synthesized during this period.

178. INTEGRATION AND EXCISION OF THE ONCOGENIC VIRUS SV40

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The objective is to understand the difference betweeen an infection by an oncogenic virus leading to a lytic cycle (in a permissive cell) and to transformation in a non-permissive cell. This problem is related to the primary and early events in the transformation phenomena.

One approach to this problem is a detailed analysis of the integration events (which occur in non-permissive cells) and the excision events (which occur upon fusion of a permissive with a non-permissive cell). As a model system, the SV40-adenovirus Ad2-HEY double plus is being used. This is a mixed population, containing adenovirus-genomes in which one or more SV40-genomes are integrated; the excision of these integrated viral genomes is being studied, as well as the factors (both cellular and viral) which affect these events. An adenovirus function which strongly interferes with SV40-DNA replication has been revealed and is to be studied further.

Recent progress has been published (a list of papers can be obtained from the principal investigator).

179. STRUCTURE AND FUNCTION OF SV40 NON-VIRION PROTEINS

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We are engaged in a coordinated study of the structure and function of the SV40 large T antigen. In progress are

experiments designed to assess 1) the role of the two enzymatic activities associated with SV40 T antigen - ATP'ase and protein kinase; 2) the nature of non-origin region SV40 DNA binding sites; 3) the role of large T in the induction of the 55K cellular Tau protein; 4) the structure of the carboxy terminus of this protein isolated from both transformed and lytically infected cells and 5) the sites of T antigen binding in cellular DNA.

BIBLIOGRAPHIC REFERENCES: Griffin, J. D., Spangler, G., and Livingston, D. M.: Protein Kinase Activity Associated with SV40 T Antigen. Proc. Nat. Acad. Sci. USA, in press, May 1979. Chang, C., Livingston, D. M., Mora, P. T., and Martin, R. G.: The T Antigen from SV40 Transformed Cells Has SV40 Tumor Specific Transplantation Antigen Activity. J. Virol. in press, 1979.

180. BIOLOGY OF SV40 SPECIFIC TRANSPLANTATION ANTIGEN

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The overall objective of the proposed research is to determine the role of specific viral genes in the expression and maintenance of the transplantation rejection antigen (TrAg) in cells infected with or transformed by papovavirus SV40. We have recently shown that a functional A gene product, which is known to be involved in the initiation and maintenance of cell transformation, is required for the expression of TrAg. We now propose to further explore the role of the viral genome in the expression of TrAg by investigating: (1) The expression of TrAg in permissive cells infected with mutants of SV40 and relationship to the map location of the mutants. (2) Expression of TrAg in tsA transformed cells and its relationship to various parameters of the transformed cell phenotype. (3) The relationship of TrAg to the A protein and to T antigen. (4) The nature of TrAg cell surface receptors. (5) Cross-reactivity between SV40 TrAg and TrAg in cells transformed by human papovaviruses. A determination of the role of SV40 genes in the expression and maintenance of TrAg and of the interaction of this antigen with the cell surface is of considerable importance in expanding understanding of viral oncogenesis and immunosurveillance mechanisms which mediate the elimination of potential tumor cells.

BIBLIOGRAPHIC REFERENCES: Tevethia, S.S., Greenfield, R.S., Flyer, D.C. and Tevethia, M.J. Simian virus 40 transplantation rejection antigen: Relationship to SV40 specific proteins. Cold Spring Harbor Symposium on Quantitative Biology, Vol. 44: in press, 1980. Tevethia, S.S. Immunology of simian virus 40. In: Viral Oncology (ed. G. Klein), Raven Press, N.Y., in press, 1980.

181. REPLICON CONTROL IN NORMAL AND TRANSFORMING CELLS

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Parvovirus and papovavirus replicons are being used as probes for the analysis of eukaryotic DNA replication and postreplication repair. The prototype linear DNA replicon is the parvovirus H-1 replicative form DNA and the mechanism of its replication is being analyzed using conditional mutants and defective genomes. Polyoma and SV40 DNA are being used as examples of circular DNA replicons and the interactions of these DNAs with parvovirus DNA are being analyzed in doubly infected cells.

BIBLIOGRAPHIC REFERENCES: Rhode, S. L. H-1 DNA Synthesis. In: Replication of Mammalian Parvoviruses. Ward, D. and P. Tattersall (eds.), Cold Spring Harbor Press, Cold Spring Harbor, NY, 1978. Singer, I. I. and S. L. Rhode. Electron Microscopy and Cytochemistry of H-1 Parvovirus Intracellular Morphogenesis. In: Replication of Mammalian Parvoviruses. Ward, D. and P. Tattersall (eds.), Cold Spring Harbor Press, Cold Spring Harbor, NY, 1978.

182. MOLECULAR BASIS OF VIRAL ONCOGENESIS

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The summary for this project is under entry no. 12.

183. MOLECULAR BIOLOGY AND GENETICS OF TUMOR VIRUSES

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The summary for this project is under entry no. 70.

3. VIRUS-HOST CELL INTERACTION

184. INTERACTION OF SV40 AND HUMAN ADENOVIRUSES - A MODEL SYSTEM TO STUDY INTEGRATION AND EXCISION OF VIRAL GENETIC MATERIAL

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OBJECTIVE: We are interested in the mechanism of integration of tumor virus DNA into the genome of mammalian cells, and its excision from the integrated state. As a model system, we have chosen to study the genetic interaction between SV40 and human adenoviruses.

APPROACH: To study integration of SV40 into the genome of adenovirus we will perform crosses between them, and select for adenovirus-SV40 hybrid viruses. Because human adenoviruses fail to replicate in monkey cells, while adenovirus-SV40 hybrids multiply efficiently, this provides a convenient method of selection for hybrid viruses. Putative hybrids will be screened for the presence of SV40 DNA sequences by hybridization, and those which contain SV40 genetic material further analyzed by restriction endonuclease cleavage and electron microscopy. By examining a large number of hybrids we should be able to determine if there is any specificity in the genetic interaction between SV40 and human adenoviruses.

Excision of SV40 DNA from the integrated state will be studied by monitoring the time course of appearance of free SV40 DNA after infection of cells with an adenovirus-SV40 hybrid population termed Ad plus HEY.: Ad plus HEY is a hybrid population which contains multiple, tandem duplications of the entire SV40 genome integrated within the adenovirus type 2 chromosome. This analysis should allow us to distinguish between two possible models for SV40 excision. If excision occurs by intramolecular recombination (Campbell model), then the first products of excision should be circular molecules of SV40 DNA, while if SV40 replicates in situ to generate free molecules as occurs in bacteriophage Mu excision (Ljungquist and Bukhari, 1977, Proc. Natl. Acad. Sci., 74, 3143-3147) then the early products will be linear molecules, and SV40 excision will depend on DNA synthesis.

PROGRESS: We have constructed a hybrid population Ad5 ts125 (Ad plus HEY) which is temperature-sensitive for adenovirus DNA replication, but not for SV40 replication. At 33 degrees C (permissive temperature) only the hybrid (Ad plus HEY) and its helper (Ad5 ts125) replicate, and very little SV40 is produced. At the restrictive temperature the hybrid and its adenovirus helper do not replicate, but SV40 is excised efficiently.

185. CHANGES IN THE DIFFERENTIATION PATHWAY AND THE TERMINALLY DIFFERENTIATED STATES OF HUMAN EPITHELIAL CELLS (ABBREV)

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OBJECTIVE: To investigate the effect of oncogenic viruses and chemicals in altering the cell proteins of human diploid epithelial cells.

APPROACH: We intend to examine the cell surface proteins and total cell proteins of diploid human keratinocytes at different stages of differentiation. We hope to be able to transform this cell type with simian virus 40 transforming genes and make comparisons of the cell proteins of normal and transformed keratinocytes. It is likely that other epithelial cell types such as liver and perhaps lung will be examined in a similar fashion during the course of these investigations. Human epithelial cells in tissue culture will be exposed to known chemical carcinogens. Any transformed cell lines generated from chemi-

cally treated cultures will be examined in parallel with virus transformed lines.

186. HOST GENE COMPONENTS IN HYBRID SV40-HOST RNA

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The proposed research will investigate the origin and function of non-viral sequences which are found at the 5' ends of early SV40 RNA transcripts, both in transformed human cells and lytically infected monkey cells. These sequences, ranging from 80 to 300 base pairs in length, have been partially sequenced by annealing a small SV40 restriction fragment to poly (A) RNA and extension of the fragment with reverse transcriptase, followed by polyacrylamide gel fractionation of the cDNA and Maxam-Gilbert procedure. Futher work will be directed at enrichment of these sequences. A synthetic oligonucleotide will be constructed which is complementary to the RNAs at the junction of viral end non-viral sequences. The oligonucleotide will be annealed to the RNA, extended with reverse transcriptase and either cloned or used directly as a hybridization probe. This probe will then be used to identify and clone the corresponding DNA template from human placental DNA. These data will reveal if and how often co-transcription of host and viral sequences occurs and may shed light on the functional mechanisms of host-viral interactions.

187. ANALYSIS OF RECOMBINATION BETWEEN SV40 DNA AND MAMMALIAN CHROMOSOMAL DNA

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We wish to describe completely the nature of the recombination events which lead to the stable association of SV40 DNA with chromosomal DNA. Furthermore, we wish to clarify the role of the SV40 gene A protein (the 96,000 dalton T-antigen) in this recombination event and in the maintenance of the transformed cell phenotype. We propose to clone, in phage lambda vectors, integrated SV40 DNA sequences together with their flanking cell sequences from various sorts of 'permissive' and 'semi-permissive' transformed cell lines. The cell sequences that serve as attachment sites for the viral DNAs will be cloned from untransformed cell DNA libraries. By a comparison of the nucleotide sequences at the viral-cell joints with those sequences which pre-exist in the chromosomal DNA, we will be able to distinguish between various sorts of recombination events. Several experiments have been described that test the notion that the replicative functions of the A gene are required for the initiation of transformation (i.e., integration) when the viral sequences are presented to the cell via viral infection as opposed to transfection. We suggest that in some of these transformed cells (SV88, CV-6, CV-2, CV-11), the replicative functions of the A gene have been lost. After cloning of the integrated DNA, this can be tested directly.

Finally, experiments have been proposed which further test our hypothesis that SV40 excision from chromosomes is mediated via an 'in situ' replication of the viral DNA sequences.

188. STUDIES ON SV40 RECOMBINANTS CONTAINING CELLULAR AND VIRAL SEQUENCES

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Improved techniques for early detection, amplification and purification of recombinants between SV40 and unique sequences in the host cell DNA have been developed. These have revealed the recurrent appearance of a particular host-substituted SV40 variant.

BIBLIOGRAPHIC REFERENCES: Winocour, E., Singer, M.F., and Kuff, E.L. Rapid detection, isolation and amplification of host-substituted SV40 variants. Cold Spring Harbor Symp. Quant. Biol., in press, Vol. 44, 1980. Singer, M.F., Kuff, E.L., Lee, T.N.H., McCutchan, T.F., Papamatheakis, J. and Thayer, R.E. A recurring SV40 variant containing monkey DNA sequences. Miami Winter Sympos., in press, 1980.

189. SV40 GENETIC ANALYSIS OF MAMMALIAN RECOMBINATION

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The objective of this research is a molecular characterization of genetic recombination processes in cultured mammalian cells. For these studies we will use an animal virus, SV40, which most likely depends on host cell enzymes for its recombination. Classical approaches to analysis of recombination with SV40 are limited in usefulness because of low frequencies of recombination. However, by constructing mixed oligomeric SV40 DNAs in vitro, we have bypassed the rate-limiting step in normal recombination with SV40 as evidenced by a 100 to 500 fold increase in measured recombination frequencies. Because SV40 DNA and these presumptive recombination intermediates are readily accessible to biochemical manipulation in vitro, we can use this sensitive genetic system to investigate molecular details of mitotic recombination in mammalian cells. By a series of two-factor crosses we propose to determine the recombinational properties of monomeric linear DNAs with a variety of end configurations. By a series of two-factor crosses using specially constructed partial dimers, we propose to measure the average size of heteroduplex segments that link recombined duplexes together. In addition we propose to develop a cell-free recombination system for the longer range goal of defining enzymatic activities and metabolites that are required for these recombination processes.

190. VIRAL CARCINOGENESIS WITH SPECIAL REFERENCE TO SV40

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The Principal Investigator has been pursuing research work pertaining to the virus-cell, virus-host and cell-host interactions in the SV40-hamster cell system, both in vitro and in vivo.

In studies of the virus-cell interaction, he has attempted to define the relative role of the viral genome virus, that of the cell genome in determining tumor-cell morphogenesis and tumor-cell differentiation, and the significance of the various virus-mediated cellular neoantigens relative to each other, to the integrated viral genome, and to the expression of a morphological and physiological state of transformation.

In relation to the virus-host interaction, he has endeavored to assess the virus-dependent (dose, route of inoculation) and host-dependent (age) factors that influence the incidence, latency and morphological variability of tumors induced by the oncogenic virus, and the significance of the development of antibody to the viral and virus-related cellular antigens in the establishment of a neoplasm in the intact host.

In regard to the cell-host interaction, he has tried to define the parameters that are responsible: for initiation of the oncogenic state, for variability of severity in oncogenic potential at the cellular level, for progression from a less to a more aggressive behavior and for the response of the host to the presence of the tumor.

191. ONCOGENIC VIRUS GENE CONTROL OF CELL GROWTH AND SHAPE

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Our long-range goal is to acquire the ability to prevent or reverse human neoplasia through clarification of the initial molecular events which underlie its appearance and progression.

Our current objectives are to understand the molecular basis of two sets of cellular changes we have observed in vitro. The first is the series of hereditable changes in growth control of rodent cells brought on by persistent expression of the integrated transforming genes of the tumor virus SV40. The second is the series of phenotypic alterations in cultured skin fibroblasts brought on by human mutations which result in inherited colon carcinoma.

Our hypothesis has been that these two sets of changes might be related in some way, for instance by being the conse-

quence of similar gene functions. In the past two years our work and that of others has led us to conclude that these two series of cellular changes are indeed functionally very similar. To the extent that this is so, any advance in our understanding of either system becomes of immediate relevance to the other. Hence, pursuit of the genetic, physiological and molecular similarities between these two in vitro systems seems to us to be a sensible immediate objective, and experiments designed to do so are described in this application. Based on these and other results, we also plan to construct culture assays to analyze the later stages of neoplasia.

BIBLIOGRAPHIC REFERENCES: Steinberg, B.M., D. Rifkin, S. Shin, C. Boone and R. Pollack. 1980. Tumorigenicity of revertants from an SV40-transformed line. J. Supramol. Struct., in press. Pollack, R., A. Lo, B. Steinberg, K. Smith, G. Blanck and M. Verderame. 1980. SV40 and cellular gene expression in the maintenance of the tumorigenic syndrome. Cold Spring Harbor Symposium 44: in press.

192. CONTROL OF HISTONE SYNTHESIS BY DNA TUMOR VIRUSES

Kellems, R. E., Baylor College of Medicine, School of Medicine, Dept. of Biochemistry, *1200 Moursund Ave., Houston, Texas*, 77025, United States of America

The research proposed here is intended to provide a better understanding of the molecular parameters underlying viral induction of cellular DNA synthesis and a group of cellular gene products related to DNA synthesis. As a model system these investigations will focus primarily on understanding polyoma and SV40 mediated regulation of histone synthesis. Several related areas of investigation will be pursued: 1) The control of histone gene expression following infection with polyoma virus, 2) the regulation of histone mRNA metabolism during abortive infection by SV40: the role of T-antigen, and 3) the relationship between virally mediated control of histone synthesis and the control by cell cycle, serum growth factors, and cyclic nucleotides. An important feature of the proposed research involves the use of cloned recombinant DNA representing the histone gene cluster and portions thereof. This will allow the use of currently available techniques to study changes in the regulation of histone gene expression (including histone mRNA metabolism) that result from polyoma or SV40 infection.

4. CELLULAR TRANSFORMATION

193. TRANSFORMATION OF BREAST EPITHELIAL CELLS BY WILD TYPE AND TEMPERATURE SENSITIVE MUTANTS OF SV40

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We have begun to isolate breast epithelial cells (usually derived from milk) transformed with wild type and temperature-sensitive mutants of SV40. We hope to obtain a range of cell lines which may represent breast epithelial cells transformed at varying stages of differentiation. Monoclonal antibodies to membrane antigens which may be differentiation-specific (see Report 166) should be useful in characterizing the cell lines. In parallel with these epithelial cell transformation studies we are also isolating SV40-transformed breast fibroblasts and myoepithelial cells. Characterization of the transformed cells and identification of the changes that occur when temperature-sensitive cells are shifted between the permissive and non-permissive temperatures may help us to understand more about the nature of malignant change in vivo and its relation to differentiation.

194. CHARACTERIZATION OF TUMOR ANTIGENS PRESENT IN SV40-TRANSFORMED MOUSE CELLS

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BALB/c 3T3 cells transformed by SV40 express, in addition to normal SV40 T-Ags, a variety of other proteins which specifically immunoprecipitate with SV40 anti-T serum. Preliminary work on the relationship of these proteins to normal SV40 T-Ags had shown that they consist of two groups. One group is closely related to normal SV40 T-Ags while the other has little or no relationship to SV40 T-Ags and is referred to as non-viral T antigen (NVT), e.g., 53K.

Several SV40-transformed cell lines have since been screened for the presence of abnormal T-Ags and we find: 1. elongated forms of large T (super T); 2. forms of large T with mobilities similar to but distinct from normal sized large-T; 3. shortened forms of large T (truncated large T MW 15-50K).

We have further characterized these proteins in some detail by two-dimensional tryptic fingerprint analysis.

All of the abnormal proteins, like normal SV40 large-T, are phosphorylated and all have the usual N terminal peptide N.Ac.Met.Asp.Lys. All the truncated large T's examined have the peptides shared by large-T and small-t; i.e., the N terminal region. In some cases no additional large-T unique peptides are detected. Super-T has the same pattern of methionine and cysteine peptides as normal large-T. No additional peptides are found; however, some peptides may be present in multiple copies. Thus there may be a duplication of part of the large-T molecule. Tentative mapping of these duplicated peptides suggests that the duplicated peptides are within 35-40K of the carboxy terminus of the large-T coding frame.

We have studied the abnormal T-Ags by cell-free translation of mRNA from these cells selected by hybridization with SV40 DNA. The pattern of proteins synthesized in vitro is virtually identical to that found by in vivo labelling. This suggests that the abnormal T-Ags are probably primary translational products and do not result from extensive post-translational modifications.

Currently we are sizing the mRNA of these proteins. This may enable us to correlate the structure of the integrated viral DNA and mRNAs with the proteins they encode.

195. ROLE OF SV40 GENE A IN CELLULAR TRANSFORMATION

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The following studies will be performed to elucidate the mechanism by which expression of the early genes of simian virus 40 (SV40) produce oncogenic transformation of mammalian cells: 1. BALB/c 3T3 mouse cells which have been transformed by tsA mutants of SV40 and which are temperature sensitive for maintenance of transformation will be analyzed to determine the role of large T antigen in producing alterations in the frequency of initiation and the temporal sequence of eukaryotic DNA replication. 2. Studies designed to generate mutants which are conditionally defective in small t protein function will be pursued to facilitate the analysis of the role of small t protein in transformation. 3. The regulation of SV40 early gene expression in the transformed state will be investigated by assessing the pattern of integration of SV40 DNA and the presence of various species of SV40 early proteins in transformed cells.

196. THE ROLE OF SV40 GENE A IN MAINTENANCE OF TRANSFORMATION

Dubbs, D. R., Baylor College of Medicine, School of Medicine, Dept. of Biochemical Virology, *1200 Moursund Ave., Houston, Texas*, 77025, United States of America

The SV40 gene A codes for three proteins, large T, small T and tumor specific transplantation antigen (TSTA). The function(s) of these proteins in maintenance of the standard transformed phenotype are unclear. mKSA207, a tsA transformant of mouse kidney cells, is uniquely suited to study this problem because: (a) it is temperature dependent for the expression of transformed growth properties; (b) it can be depleted of intranuclear T antigen, and (c) at the nonpermissive temperature (39.8 degrees) newly synthesized T antigen accumulates in the cytoplasm. Cytoplasmic T antigen will be extracted from mKSA207 cells at 39.8 degrees, partially purified, and characterized as to: (a) size in SDS-polyacrylamide gels; (b) state of aggregation; and (c) heat stability of TSTA, T antigenicity, and DNA binding capacity. Nuclear extracts of mKSA207 at 39.8 degrees are being studied to see whether any gene A products are transported to the nucleus. TSTA activity is being assayed by tumor rejection test using a transplantable SV40 tumor line. Cytosol, nuclear, and membrane fractions from mKSA207 at 39.8 degrees are being studied to learn whether TSTA activity is associated with cytoplasmic T antigen, is transported to the nucleus, or is translocated to the membrane. Results obtained

with mKSA207 are being compared with those obtained with heat-adapted mKSA207 cells which are temperature independent for expression of transformed phenotype but still display cytoplasmic T antigen at 39.8 degrees.

197. TRANSFORMATION GENES OF SIMIAN VIRUS 40

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The role (and even the number) of the early region functions involved when SV40 transforms a cell is unresolved and complex. Efficient transformation requires small t; yet transformation can occur at low frequency without it. Large T is also required in some clones to maintain the transformed state; yet in other cases it is not required. We have begun to analyze a new mutant in the early region with novel effects on transformation not predicted by known large T or small t effects.

We plan to continue the in-depth analysis of these three functional types of mutants and their interactions by clonal analysis, protein analysis, copy number and rescue and testing of transforming genomes. We will attempt to generate a ts small t mutant which should then provide us with conditional mutants for all three SV40 functions which have been implicated in viral transformation and a unique opportunity to determine which of these functions are crucial to the maintenance of viral dependent transformation.

198. CELL SURFACE CHANGES IN SPONTANEOUSLY OR SV40 TRANSFORMED MOUSE CELL LINES

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The purpose of this project is to study the cell surface membrane -how it may change in transformation to malignancy either by spontaneous induction or as induced by SV40 virus; to study the nature of tumor antigens associated with cells transformed by either SV40, chemicals, or spontaneous induction; and the relation of these antigens to gene expression and host immune mechanisms. Research is carried out on: 1) Changes in glycoconjugates, including glycoproteins and sulfated glycosaminoglycans such as heparan sulfate, and their correlation with cell growth properties in tissue culture and in vivo, in the syngeneic and in the nude mouse; 2) controls of expression and methods of purification of SV40 specific T antigens, surface antigens and tumor specific transplantation antigens (TSTA); 3) the relationship between SV40 T, surface and TSTA antigens, and their relationship to SV40 A gene expression; 4) a 56,000 MW SV40 induced but cell DNA encoded protein which is also present in embryonal carcinoma cells and in certain embryo cells; 5) study of 'spontaneous' transformation of cells in culture and of the balance between such tumorigenicity and the SV40 TSTA in families of clonal cells with known genealogy.

BIBLIOGRAPHIC REFERENCES: Simmons, D. T., Martin, M. A., Mora, P. T., and Chang, C.: Relationship among TAU antigens isolated from various lines of simian virus 40-transformed cells. J. Virol., 1980, in press. Chandrasekaran, K., Winterbourne, D. J., Luborsky, S. W., and Mora, P. T.: Surface proteins of simian virus 40 transformed cells. Virology, 1980, in press.

199. EVALUATION OF THE PROCESS OF VIRAL INTEGRATION DURING TRANSFORMING INFECTION BY SV40

Botchan, M. R., Kriegler, M. P., University of California, Berkeley Campus, School of Letters & Science, Dept. of Molecular Biology, *Berkeley, California,* 94720, United States of America

The objective of my proposed research is to elucidate the biochemical and molecular genetic mechanism leading to the initiation of transformation of mammalian cells by simian virus 40.

Full copies of viral DNA are found integrated into the host chromosomal DNA of many SV40 transformed cell lines and infectious virus can be rescued from these cells. Integration of the entire viral genome is not mandatory for transformation because cells can be transformed by fragments obtained by digestion of viral DNA by restriction endonucleases.

SV40 tsA mutants are ts in the initiation of viral DNA synthesis. Infection of non-permissive cells with SV40 tsA mutants reveals a strong effect of this mutation in neoplastic transformation. Certain A mutant transformants generated at the permissive temperature regain certain elements of normal growth control when grown at high temperature.

There is little information about the molecular events that comprise the initiation of transformation. Is the A gene product necessary for viral integration? Does the viral DNA replicate before viral integration? What is the state of the viral DNA immediately after integration - before the onset of cell division? Does the observed fragmentation of the integrated SV40 occur before or after integration? I propose to trace the integration of the viral genome during transforming infection. I will assay for integration of the viral genome by 'Southern blot' analysis and, where necessary, molecular cloning of the DNA of interest in a bacterial vector followed by restriction enzyme mapping and DNA sequence analysis. These experiments will be performed on cultures: 1) Infected with SV40 tsA mutants at the permissive and non-permissive temperatures, to determine the role of the A gene during integration; 2) co-infected with the SV40 tsA Bam/Hpa2 fragment and the cloned HSV thymidine kinase gene (either separately or in a covalently coupled unit), to determine if the A gene effects the manner in which integration occurs; 3) infected with replication minus SV40 mutants cloned in a bacterial vector, to determine if viral DNA synthesis is required for integration.

200. SV40 TRANSFORMATION OF GARDNER'S SYNDROME FIBROBLASTS

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Our work on heritable forms of cancer in man suggests than 'non-tumor' cutaneous cells can be utilized for diagnosis of clinically latent manifestations of neoplasia. Abnormal phenotypic expressions have been identified in cultured skin fibroblasts (SF) derived from normal-appearing skin biopsies of tumor-prone and tumor-bearing individuals with hereditary adenomatosis of the colon and rectum (ACR). These consisted of growth disorders, increased proteolytic activity, cytoskeletal alterations, and increased susceptibility to transformation by the Kirsten murine sarcoma virus. We have proposed that the ACR cell exists in an initiated state due to a dominant mutation. Apparently, systemic disorders occurring within stromal cells of individuals at high risk of developing cancer are intrinsically linked to cancer and will be used in studies concerning cancer causation and cancer progression. The present report concerns: 1) The apparent susceptibility of ACR cells to an SV40-induced T-antigen display and transformation; 2) the ability of SF derived from normal and ACR individuals to repair irradiation-induced defective SV40 virus functions in infected cells; 3) the expression of neoantigens by skin fibroblasts derived from normal and ACR individuals prior to and following infection by DNA and RNA oncogenic viruses; and 4) the effects of SV40 and 12-0-tetradecanoyl phorbol-13-acetate (TPA) on the phenotypic profile of ACR cells.

BIBLIOGRAPHIC REFERENCES: Kopelovich, L. Familial polyposis: A model of tumor progression and tumor prognosis. Surgical Rounds 3:30-34, 1979. Kopelovich, L., Bias, N. and Helson, L. Tumor promoter alone induces neoplastic transformation of fibroblasts from humans genetically predisposed to cancer. Nature 282:619-621, 1979.

201. HOST CELL FUNCTIONS INVOLVED IN SV40 TRANSFORMATION

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Experiments are proposed to study the involvement of host cell DNA repair functions in cellular transformation by simian virus 40 (SV40). Transformation of the following repair-deficient cells will be studied: human cells carrying known or potential repair deficiencies (xeroderma pigmentosum, Bloom's syndrome, Fanconi's anemia), primary baby mouse kidney cells unable to repair ultraviolet(UV)-irradiated polyoma virus, and

mouse cells selected in culture for altered responses to UV light or mitomycin C. The involvement of repair functions in transformation will be determined by measuring transformation frequencies, the frequency of integration of viral DNA into the host genome, and the frequency of virus rescue (excision) following transformation with untreated virus or virus inactivated with UV light or mitomycin C. In order to determine whether the action of repair functions early in transformation can affect the ultimate phenotype of the transformed cells, tumor(T) antigen levels and cellular growth properties of isolated transformed cells will be studied. Experiments will also be performed to elucidate the mechanism of viral transformation events involving host DNA repair functions.

202. VIRAL INDUCTION IN SV40-TRANSFORMED HUMAN CELLS

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A study of viral induction in SV40-transformed human cells is proposed. The project is divided into three stages: 1. To transform Lesch-Nyan fibroblast cells with ts-A mutants of SV40 and to confirm viral induction in these cell lines. 2. To characterize the physical structures of integrated and free viral DNA in transformed cells, with the purpose of correlating the efficiency of viral induction and the structure of excised viral genomes to the structure of the integrated array. 3. To identify human chromosomes involved in the regulation of the inducible state of the transformed cells, by selecting SV40-transformed human cell and mouse cell hybrids and correlating karyotypes of these hybrid cells to their ability to produce viruses. Chromosomes that have viral genomes integrated as well as those that carry genes necessary for viral replication could be identified.

The experimental system proposed above could provide a controlled condition for studying mechanism of viral induction in SV40-transformed cells.

203. GENETICS OF SV40 VIRAL TRANSFORMATION OF HUMAN CELLS

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Simian virus 40 (SV40) transformation provides an in vitro model for cell changes accompanying viral oncogenesis. The frequency of this event varies with the cell line studied. Past studies have indicated that certain human cell lines (including those from patients with Down's syndrome and Fanconi's anemia) have an increased susceptibility to transformation by SV40. Clinical data show that these patients also have an increased incidence of various types of cancer. I plan to reexamine these observations in the cell lines already studied as well as in others (including xeroderma pigmentosum, Bloom's, Gardner's, Peutz-Jeghers and the basal cell nevus syndromes as well as other stable trisomies and translocations). The criteria for identification of transformation will include: altered colonial morphology, presence of SV40 viral T antigen, ability to grow in soft agar, and fibrinolysis. If the observation can be confirmed that the differences in transformation susceptibility are eliminated by infection with isolated viral DNA, I propose study of adsorption of viral particles by the cell lines. The adsorption assay will measure the efficiency of binding of infectious particles to the cells rather than radioactively labeled particles which may not be all biologically active. The intracellular fate of SV40 will be studied both by measuring the deoxyribonuclease susceptibility of labeled viral DNA and by using viral mutants with structural proteins either altered (e.g., tsD mutant) or absent (e.g., deletion mutants) which are known to have effects on uncoating and/or infection. Finally, hybridization of 'low' and 'high' susceptibility cell lines can show whether susceptibility to SV40 viral transformation involves factors which have dominant function in a heterokaryon. In addition, chromosome study of such cells may help to identify the location of critical cellular genes affecting the process.

204. SV40 VIRUS-INDUCED CARCINOGENESIS - STRUCTURE OF THE VIRAL GENOME AND THE MECHANISM OF CELLULAR TRANSFORMATION

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The summary for this project is under entry no. 105.

205. CONTROL OF GENE EXPRESSION IN HUMAN CELLS TRANSFORMED BY SV40 VIRUS

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OBJECTIVE: To elucidate the role of small molecular weight nuclear RNA (SnRNA) in the onset and maintenance of the transformed phenotype in SV40-transformed WI38 cells.

APPROACH: SnRNA's are extracted from the chromatin of normal and transformed cells with 0.35 M NaCl and fractionated in polyacrylamide gradient slab gels. Purified subspecies are then extracted from gel slices and tested as to their activity on transcription of isolated homologous and heterologous nuclei in vitro using endogenous and E. coli RNA polymerase. The 'active' bands are further characterized as to their origin, mode of action and sequence.

PROGRESS: Work to date supports the concept that a subset of SnRNA participates in the regulation of gene transcription by recognizing base sequence in promoter or regulator regions of DNA. Although we have found tissue-specific 'active' RNA's in normal human and monkey cells, the properties of the RNA from SV40-transformed human cells clearly differentiate it from its nontransformed counterparts, i.e., its stimulatory activity on transcription is higher than that of normal cell SnRNA and it is the only kind of SnRNA which is capable of activating transcription in the nuclei of an heterologous species (monkey).

206. EVALUATION OF THE PROCESS OF VIRAL INTEGRATION DURING TRANSFORMING INFECTION BY SV40

Kriegler, M. P., University of California, Berkeley Campus, School of Letters & Science, Dept. of Molecular Biology, *Berkeley, California*, 94720, United States of America

The objective of my proposed research is to elucidate the biochemical and molecular genetic mechanism leading to the initiation of transformation of mammalian cells by simian virus 40.

Full copies of viral DNA are found integrated into the host chromosomal DNA of many SV40 transformed cell lines and infectious virus can be rescued from these cells. Integration of the entire viral genome is not mandatory for transformation because cells can be transformed by fragments obtained by digestion of viral DNA by restriction endonucleases.

SV40 tsA mutants are ts in the initiation of viral DNA synthesis. Infection of non-permissive cells with SV40 tsA mutants reveals a strong effect of this mutation in neoplastic transformation. Certain A mutant transformants generated at the permissive temperature regain certain elements of normal growth control when grown at high temperature.

There is little information about the molecular events that comprise the initiation of transformation. Is the A gene product necessary for viral integration? Does the viral DNA replicate before viral integration? What is the state of the viral DNA immediately after integration - before the onset of cell division? Does the observed fragmentation of the integrated SV40 genome occur before or after integration? I propose to trace the integration of the viral genome during transforming infection. I will assay for integration of the viral genome by 'Southern blot' analysis and, where necessary, molecular cloning of the DNA of interest in a bacterial vector followed by restriction enzyme mapping and DNA sequence analysis. These experiments will be performed on cultures: 1) Infected with SV40 tsA mutants at the permissive and non-permissive temperatures, to determine the role of the A gene during integration; 2) co-infected with the SV40 tsA Bam/Hpa2 fragment and the cloned HSV thymidine kinase gene (either separately or in a covalently coupled unit), to determine if the A gene effects the manner in which integration occurs; 3) infected with replication minus SV40 mu-

tants cloned in a bacterial vector, to determine if viral DNA synthesis is required for integration.

207. BIOCHEMICAL STUDIES ON T ANTIGEN AND TRANSFORMED CELLS

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Simian virus tumor (T) antigen will be compared to the adeno-SV40 hybrid T antigen with respect to single and double strand DNA binding, site specificity and catalytic activities. The poly dT stimulated ATPase activity of T antigen will be defined in terms of the minimum size of the poly dT molecule required for optimum activation of the ATPase activity. The high affinity binding of poly dT to the T antigen will also be measured. The relation of the ATPase activity of T antigen to its role in the initiation of DNA synthesis will be examined. Attempts will be made to assay 'in vitro' initiation of SV40 viral DNA in the presence of purified T antigen, host polymerases and other factors required for eucaryotic DNA synthesis. Attempts will be made to convert transformed cells to normal cells by competing T antigen off cellular DNA and onto poly dT or other high affinity binding single strand DNA species.

208. SIMIAN VIRUS 40, DNA REPLICATION AND TRANSFORMATION

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The summary for this project is under entry no. 169.

209. BIOSYNTHESIS IN NORMAL AND VIRUS-TRANSFORMED CELLS

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The summary for this project is under entry no. 155.

210. CELLULAR AND VIRAL CONTROL OF ONCOGENIC TRANSFORMATION

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The objective of this research is to study the viral and the cellular control of the transformed phenotype in cells transformed by the oncogenic viruses polyoma and SV40. We also want to study the control of viral DNA integration and of the expression of viral functions in the transformed cells.

Our work is planned along three main lines of research: 1) The study of viral and cellular factors controlling the association between the viral and the cellular genome in rat cells transformed by polyoma virus. In this system, 'free' and integrated viral DNA molecules coexist, and the free molecules result from excision and limited replication of the integrated viral DNA. Excision requires an active viral A-gene product and generates 'cured' cells which have lost an integral number of viral DNA molecules. Amplification of integrated viral DNA can also occur in the presence of a functional large T antigen. 2) The study of the regulation of viral transcription and T antigen expression in SV40 transformed cells. Expression of viral functions appears in fact to be controlled by the position of the transformed cell in the mitotic cycle. 3) The study of the minimal amount of viral functions required for cell transformation by polyoma virus.

211. MECHANISMS OF TRANSFORMATION BY ONCOGENIC VIRUSES

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The summary for this project is under entry no. 100.

212. PATHOLOGY OF NEOPLASTIC TRANSFORMATION

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This proposal will continue the analysis of the mechanism of transformation with the papovavirus, simian virus 40. We have shown that the stem cell (embryonal carcinoma) of the teratocarcinoma, when infected with SV40 or polyoma virus, does not express viral function (T antigen, V antigen, or infectious virus). This work will be continued by analyzing the presence or absence of the viral DNA, the state of the viral genome, whether the DNA is transcribed, and whether small amounts of viral protein are detectable by techniques other than immunofluorescence. These studies will be useful in defining how the state of differentiation may modulate virus infection and transformation.

In the second series of questions, we propose to analyze the virus-cell interactions required for infection and transformation of diploid Chinese hamster cells with SV40 virus. Early after infection, a population (30%) of tetraploid-polyploid cells is induced, which we propose to separate with flow cytometry. We propose to further characterize this population in regard to the acquisition of their malignant phenotype. Further studies are proposed to characterize the mechanism of cell death in populations of SV40 tsA-transformed Chinese hamster cells at the nonpermissive temperature. Various analyses, such as immunoprecipitation of T antigen, analysis of the state of the viral DNA, cell cycle regulation, and certain transformed cell cycle markers are proposed.

Finally, we plan to test the tumorigenicity of SV40 tsA-transformed cells in goats (39.5 degrees C temperature), and in the chorioallantoic membrane of the chicken egg.

These studies will provide information to aid in understanding the mechanism of viral infection and transformation.

213. REGULATION OF DNA SYNTHESIS IN VIRUS-TRANSFORMED CELLS

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OBJECTIVE: To analyze at the molecular level the control of cell growth (transition from G0 to G1 and back) in fibroblasts growing in vitro.

APPROACH: Expression of the A gene of polyoma and SV 40 viruses is followed by induction of cell division and DNA synthesis in resting normal cells and permanently transformed cells. The function of this protein is being analyzed: a) at the molecular level: the A gene product carrying the T antigenic specificity is purified on this basis in a native form and its function is analyzed using wild-type and mutant (ts, deletions) gene products; b) at the cellular level: analysis of the transformation parameters (saturation density, cloning ability, anchorage dependence, deoxyglucose uptake, plasminogen activator production) of cells either acutely infected or transformed by wild-type and mutant virus.

214. EXPRESSION AND MODULATION OF THE SYNTHESIS OF ESTERASES DURING IN VITRO DIFFERENTIATION OF TRANSFORMED CELLS (ABBREV)

Lanciem, M., Universita degli Studi di Napoli, Istituto di Biologia Generale e Genetica, *Via Mezzocannone 8, 80134 Naples, Italy*

OBJECTIVE: Polyoma virus and SV40 can abolish the growth control of cells 'in vitro' and these virus-transformed cells acquire a set of properties, one of which causes tumors when cells are injected into susceptible animals. Previous results have shown that the interaction of viral genome with Friend erythroleukemic cells (FLC) causes various phenotypic expressions and alters their differentiation capability towards different types of inducers.

APPROACH: We should like to study the biochemical changes produced in the FLC by polyoma and SV40 infection and we want to determine whether the differentiation capability can be correlated to the alterations of esterase pattern, as the oncogenic transformation causes in the sublines of 3T3 cells some gradual alterations of esterase pattern and the reappearance of enzyme forms of embryonic tissues.

PROGRESS: Lines of persistently infected FLC were established after polyoma infection. Among 20 isolated clones analyzed, clones 9, 13 and 18 were found to be producing low, intermediate and high percentages of T and V Py antigens and virus particles. Cells from the three clones were compared with uninfected FLC for their esterase content using differential sub-

strates. The results indicate a net alteration of the esterase pattern in all three clones, compared to FLC, and might indicate that Py persistence induces the appearance of some esterase variants as well as the quantitative alterations of those observed in uninfected cells.

215. KARYOTYPIC AND PHENOTYPIC CHANGES IN THE PROGRESSION OF VIRALLY TRANSFORMED CELL LINES (ABBREV)

Gallimore, P. H., University of Birmingham, School of Medicine & Dentistry, Dept. of Cancer Studies, *St. Chads Queensway, Birmingham B4 6NN, England, United Kingdom*

OBJECTIVE: To build on our knowledge of the events and changes occurring during the process of virus induced cell transformation and to study the selective influences involved in malignant progress of virus transformed cells.

APPROACH: Mammalian cells (both fibroblastic and epithelial) are transformed in vitro by purified virus or the transformation genes of adenovirus 2, 12 or simian virus 40. A number of randomly selected transformed foci representing unique transformation events are examined for the expression of traits generally associated with transformation. Each transformed cell line is G-banded and karyotyped. Assessment is made of the oncogenic potential of each transformation event in immunocompetent and immunosuppressed syngeneic rats or in congenitally athymic nude mice.

A comparison will be made of the in vitro and in vivo findings with special attention being paid to traits associated with invasion of local tissues and metastatic spread. The importance of the immunological status of the host, the site of inoculation and the antigenicity of each transformed cell line to tumor progression will be studied.

PROGRESS: Virus transformed cells do not always proliferate in vivo. Indeed virus transformed cell lines cover the spectrum from showing no in vivo growth in nude mice through being highly malignant in adult syngeneic rats. No single in vitro characteristic has been found to equate with malignant behavior. Virus transformed cell lines are not always karyotypically abnormal. No specific virus induced chromosome changes have thus far been observed.

216. INTEGRATION AND EXCISION OF THE ONCOGENIC VIRUS SV40

Fiers, W., Van Roy, F., Rijksuniversiteit Te Gent, Molecular Biology Lab., *Ledeganckstraat 35, B-9000, Ghent, Belgium*

The summary for this project is under entry no. 178.

217. CHARACTERIZATION OF THE C-TERMINAL REGION OF SV40 T-ANTIGEN

Denhardt, D. T., Crawford, L. V., Imperial Cancer Research Fund, Molecular Virology Laboratory, *Lincolns Inn. Fields, London WC2A 3PX, England, United Kingdom*

The summary for this project is under entry no. 176.

218. TEMPERATURE-SENSITIVE INDUCTION AND ASSOCIATION OF A LARGE 53K PHOSPHOPROTEIN WITH LARGE-T ON 3T3 CELLS INFECTED WITH SV40 TSA30

McCormick, F., Imperial Cancer Research Fund, Translation Laboratory, *Lincolns Inn Fields, London WC2A 3PX, England, United Kingdom*

The summary for this project is under entry no. 177.

219. MECHANISMS OF VIRAL ONCOGENESIS - SV40 PROTEIN FUNCTION

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SV40 encodes at least two transforming proteins: the 94K A protein (large T antigen) and the 17K F protein (small T antigen). The A protein regulates the initiation of viral DNA replication and transcription and is required to maintain all aspects of the transformed phenotype in some cell types. The F protein has no known function in productive infection and is required for the expression of a defined subset of the transformed phenotype including anchorage-independent growth and cytoskeletal structure.

We plan to study the structure and function of the A and F proteins in vitro and to detect structural or enzymatic interactions between the viral transforming proteins and specific cellular proteins.

220. SV40 EARLY PROTEINS - POSSIBLE ROLES IN ONCOGENESIS

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Simian virus 40 (SV40) can cause tumors when injected into susceptible hosts. We propose to isolate new SV40 mutants which are defective in the genes coding for two tumor antigens, t and T. These new mutants will be used to study the role of these putative oncogenic proteins in cell transformation by SV40 and in oncogenesis. Rat cells transformed by new mutants with genotypes t negative Tts and tts T negative will be used to determine which transformed cell phenotypes are under the maintenance control of t or T. Immunocompatible rats will be inoculated with rat cells transformed by mutants with genotypes t negative T positive and t positive T negative to determine whether t or T (or both) is involved in SV40 oncogenesis. Finally, these mutants will be used to study the mechanisms by which these putative proteins may act to cause cell transformation and tumors.

We have isolated 159 mutants with deletions in the gene for t (but not T) ranging in size from 3% to 5% of the genome. These have been characterized and we have selected 29 mutants that have the lowest efficiency of transformation of cells to grow in soft agar. We are screening these for temperature-sensitivity.

We have constructed several mutants with large deletions in the distal end of the gene for T and are characterizing the genomes of these mutants by fine structure restriction enzyme mapping and DNA sequence analysis. We are testing these mutants to determine if a normal t protein is made and whether T antigen, albeit truncated, is present in infected cells.

We have constructed a t negative Tts double mutant by joining together the defective regions of two existing mutants, dl884 and tsA58. This mutant does not produce a detectable t protein but does produce a thermolabile T antigen of normal size. We are using this mutant in transformation studies.

We are attempting to isolate a ttsT positive mutant by mutagenizing the t-specific region of the genome; we are characterizing all of our mutants, and will use them during the second two years of grant period.

221. ORIGIN AND FUNCTION OF SV40-SPECIFIC TAU ANTIGENS

Simmons, D. T., University of Delaware, School of Arts & Sciences, Dept. of Cell & Molecular Biology, *Newark, Delaware*, 19711, United States of America

We have been investigating the properties of a group of proteins called Tau antigens that can be isolated from various lines of SV40-transformed cells by immunoprecipitation with serum obtained from hamsters bearing SV40-induced tumors (anti-T serum). Work in several laboratories has shown that these proteins can be isolated from mouse embryonal carcinoma cells and from cells transformed by various retroviruses, papovaviruses, and chemicals. They have not been detected in significant amounts in normal, untransformed cells or in tumor antigen-negative revertant cell lines derived from SV40-transformed mouse or rat cells.

We have determined the relationship between Tau antigens isolated from various lines of SV40-transformed cells by comparing their methionine-containing tryptic peptides by two-dimensional fingerprinting. All Tau antigens studied so far appear to share some common peptides. Those isolated from closely related species (i.e., mouse and rat) show the greatest similarity. Within a certain species (mouse, hamster), the Tau antigens are very closely related or perhaps identical.

Recently, we have detected Tau antigen in monkey cells infected with SV40. This protein is closely related to the Tau antigen isolated from a line of human cells transformed by SV40.

Finally, we have compared the Tau antigens isolated from hamster cells transformed by different primate papovaviruses. In this study, we showed that the peptide fingerprints of the protein isolated from cells transformed by SV40, BK and JC viruses were indistinguishable.

BIBLIOGRAPHIC REFERENCES: Israel, M.A., D.T. Simmons, S.L. Hourihan; W.P. Rowe, and M.A. Martin. 1979. Proc. Natl. Acad. Sci. U.S.A. 76:3713-3716. Simmons, D.T., M.A. Martin, P.T. Mora, and C. Chang. 1980. J. Virol. in press.

222. CHARACTERIZATION OF THE MIDDLE T ANTIGENS OF SV40

Livingston, D. M., Kilton, L., Sidney Farber Cancer Center Inc., *44 Binney St., Boston, Massachusetts*, 02115, United States of America

There now appear to be multiple SV40 tumor antigens - large T, small T, and one or more 'middle' T antigens. A role in the virus-induced neoplastic transformation process has been assigned to large and small T. To date, we know little that would suggest a role for SV40 middle T antigens, but they do exist for polyoma virus as well, and at least one has been found for association with the cell surface in that system. In the case of SV40, they appear, thus far, to be present in transformed cells and from preliminary qualitative peptide map studies do not appear to be simply cleavage products of large T. This information does suggest that these proteins are, to a limited degree, subsets of large and small T and in part unique. They might therefore result from splicing (in the messengers) of regions of the T antigen coding segments with either host cell sequences or with other presumably early region segments which are large and/or small T antigen coding segments but are now read in another frame to give new polypeptide sequences.

I will attempt to purify these polypeptides in denatured form from one or more SV40 transformants by selective immunoprecipitation and preparative SDS gel electrophoresis. The isolated polypeptides - once obtained in pure form - will be subjected to one or more methods of cleavage. I will seek a cleavage method which results in a small number of large peptides which can be readily separated into pure species. Each peptide will then be sized and subjected to N- and C-terminal analysis, N- and C-terminal sequence analysis, and amino acid analysis. By correlating this information with the known sequence of SV40 DNA, it should be possible to derive useful information on the location and organization of the coding regions for these proteins.

223. BIOCHEMICAL PROPERTIES OF THE SV40 T ANTIGENS

Carroll, R. B., Melero, J. A., Greenspan, D. S., New York University, School of Medicine, Dept. of Pathology, *550 1st Ave., New York, New York*, 10016, United States of America

The early or transforming region of simian virus 40 encodes the T Ags (tumor antigens) and induces TSTA (the tumor specific transplantation antigen). The primary objectives of this research are threefold: 1) To study by physical methods and affinity columns key reactions of large T Ag (self-association, nucleotide and DNA binding, and interaction with the new 48K-55K phosphoprotein species) which are important to understanding its probable enzymatic activity in initiating DNA synthesis; (2) to characterize the new 48K-55K phosphoprotein species (isolated in my laboratory) by a) mRNA isolation and in vitro translation and b) preparation of specific antisera to determine whether they are either 1) virus coded or 2) host coded, but virus-induced or modified; and 3) to characterize the new putative cell-surface (PCS) glycoprotein species recently isolated in my laboratory, which crossreacts with large T Ag by comparison of its tryptic fingerprint with large T Ag. In addition we will determine by immunization with the isolated PCS whether it is related to TSTA, the viral antigen mediating tumor rejection. Unique to this work has been our use of antisera to pure large T Ag to isolate new species which may be the postulated viral products such as TSTA.

BIBLIOGRAPHIC REFERENCES: Carroll, R.B., Goldfine, S.M., and Melero, J.A. Antiserum to Polyacrylamide Gel-Purified Simian Virus 40 T Antigen. Virology 87: 194 (1978).

224. THE SV40 TUMOR ANTIGEN

Tjian, R. T., Robbins, A., Myers, R., University of California, Berkeley Campus, School of Letters & Science, Dept. of Biochemistry, *Berkeley, California*, 94720, United States of America

We are using a battery of biochemical techniques as well as recombinant DNA methods to determine how the large T-antigen of SV40 regulates gene expression and effects neoplastic transformation.

Our studies indicate that large T-antigen interacts with the major groove of the SV40 DNA at three tandem binding sites in a sequential manner. More recently, we have shown that large T-antigen is an active enzyme capable of hydrolyzing ATP at a rate of 0.6 micromoles/hr/mg. The T-antigen ATPase is rapidly inhibited by monoclonal antibodies directed against large T and is greatly stimulated by poly d T. Moreover, homogeneously pure large T-antigen is able to bind specifically to a 54,000 dalton cellular phosphoprotein found in transformed cells but not detectable in normal cells. Finally, we have shown that small amounts of purified T-antigen can stimulate the production of sensitized lymphocytes capable of specifically killing SV40 transformed cells in vitro.

225. SV40 EARLY GENE PRODUCTS AND VIRAL ONCOGENESIS

Topp, W. C., Cold Spring Harbor Lab. of Quantitative Biology, Tumor Virology Section, *P.O. Box 100, Cold Spring Harbor, New York*, 11724, United States of America

The small papovavirus SV40 induces tumors in experimental animals, and transforms cells in culture. Preliminary experiments have shown that only the early region of the SV40 genome contributes to transformation. Both gene products of the SV40 early region (small-t and large-T proteins) as well as the viral origin of early transcription, may be required.

Viral mutants defective in large-T (TsA mutants) or in small-t (dl 54/59 mutants) exist. Using rat and hamster embryo fibroblasts transformed by wild type SV40 and by these mutants we will attempt to link the various changes associated with the transformed state (e.g., in growth properties, in synthesis of plasminogen activator, in metabolic state and in the ability to form tumors in nude mice) with the presence of functional small-t or large-T antigen. We will select from rat embryo fibroblast transformants revertants which have lost specific aspects of the transformed phenotype and attempt to correlate this reversion with specific alterations in the integrated viral genome.

We now know that certain of the dl 54/59 mutants (e.g., 2007) may be partially leaky for some small-t gene function. We will characterize cells transformed by these mutants and compare the phenotype to cells transformed by WT virus and by the tight mutants. We will also proceed with the isolation of TsA/tight dl 54/59 double mutants and will characterize cells transformed by these viruses.

We have found that the dl 54/59 viruses are tumorigenic but that the effect of the presence of small-t is to increase the incidence and decrease the latency of tumors. We will carry out experiments to test whether small-t may be acting by a mechanism similar to those proposed for tumor promoters such as PMA.

Finally we will proceed with the isolation of control mutants of SV40 lacking sequences important for early message transcription or processing.

226. CHARACTERIZATION OF THE MIDDLE T ANTIGENS OF SV40

Kilton, L., Sidney Farber Cancer Center Inc., Division of Medical Oncology, *44 Binney St., Boston, Massachusetts*, 02115, United States of America

There now appear to be multiple SV40 tumor antigens - large T, small T, and one or more 'middle' T antigens. A role in the virus-induced neoplastic transformation process has been assigned to large and small T. To date, we know little that would suggest a role for SV40 middle T antigens, but they do exist for polyoma virus, as well, and at least one has been found in association with the cell surface in that system. In the case of SV40, they appear, thus far, to be present in transformed cells and from preliminary qualitative peptide map studies do not appear to be simply cleavage products of large T. This information does suggest that these proteins are, to a limited degree,

subsets of large and small T and in part unique. They might therefore result from splicing (in the messengers) of regions of the T antigen coding segments with either host cell sequences or with other presumably early region segments which are large and/or small T antigen coding segments but are now read in another frame to give new polypeptide sequences.

I will attempt to purify these polypeptides in denatured form from one or more SV40 transformants by selective immunoprecipitation and preparative SDS gel electrophoresis. The isolated polypeptides - once obtained in pure form - will be subjected to one or more methods of cleavage. I will seek a cleavage method which results in a small number of large peptides which can be readily separated into pure species. Each peptide would then be sized and subjected to N- and C-terminal analysis, N- and C-terminal sequence analysis, and amino acid analysis. By correlating this information with the known sequence of SV40 DNA, it should be possible to derive useful information on the location and organization of the coding regions for these proteins.

227. CHARACTERIZATION OF SV40-SPECIFIC MEMBRANE ANTIGENS

SchmidtUllrich, R., Lin, P., Mikkelsen, R. B., Wallach, D. F., Tufts University, Boston Campus, New England Medical Center Hospital, Dept. of Therapeutic Radiology, *171 Harrison Ave., Boston, Massachusetts*, 02111, United States of America

The long-term project is to characterize structure-function relationships of the two simian virus 40 (SV40)-specific membrane antigens defined as surface (S) and perinuclear (U) antigen. In these studies initially SV40-transformed lymphocytes (GD248) will be used; later we will also employ a human SV40-transformed lymphoid cell line. The projects planned can be subdivided into three major categories: 1. Quantitative distribution of the two antigens in different subcellular membranes. 2. Spacial distribution and neighborhood relationships of these antigens within the membrane. 3. Their chemical characterization in different membranes to assess the mechanisms of host cell modification of one original SV40 gene product. Working with lymphoid cells neoplastically transformed with SV40 we plan: (a) to establish a human lymphoid cell line derived from T- or B-cells transformed in vitro with SV40; (b) to prove whether both S- and U-antigens are expressed on the cell surface; (c) to define their quantitative distribution in the outer envelope of the nuclear membrane (domain of U-antigen), in membranes of the endoplasmic reticulum and in the plasma membrane (domain of S-antigen); (d) to analyze the spatial distribution of S- and U-antigens across the membrane using controlled proteolysis and labeling techniques from both sides of the membrane; (e) to specifically investigate the neighborhood relationships between SV40 and HL-A antigens in plasma membranes of human lymphoid cells using (1) hydrophilic and hydrophobic photoactivated reversible cross-linking agents (2) followed by immunochemical analyses of cross-linked membrane proteins/glycoproteins with sera specific for HL-A, S- and U-antigens; (f) to isolate SV40-induced S- and U-antigens by preparative isoelectric focusing on the basis of their extreme isoelectric points, pI 4.5 and pI 4.7 respectively; (g) to chemically characterize the protein and carbohydrate moieties of the two groups of antigens using (1) N-terminal amino acid analyses (2) peptide/glycopeptide mapping, (3) amino acid analyses of peptides (glycopeptides), and (4) bulk and sequential analyses of the oligosaccharide residues.

228. SV40 T ANTIGEN BINDING SITES IN CELLULAR DNA

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The summary for this project is under entry no. 158.

229. FUNCTION AND EXPRESSION OF SV40 VIRAL TUMOR ANTIGENS

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The summary for this project is under entry no. 150.

230. BIOLOGY OF SV40 SPECIFIC TRANSPLANTATION ANTIGEN

Tevethia, S. S., Tevethia, M. J., Pennsylvania State University, Milton S. Hershey Medical Center, School of Medicine, Dept. of Microbiology, *500 University Dr., Hershey, Pennsylvania*, 17033, United States of America

The summary for this project is under entry no. 180.

231. STUDIES ON THE SV40 T-ANTIGEN BINDING SITE

Caruthers, M. H., University of Colorado, Boulder Campus, School of Arts & Sciences, Dept. of Chemistry, *1200 University Ave., Boulder, Colorado*, 80302, United States of America

The summary for this project is under entry no. 159.

232. STRUCTURE AND FUNCTION OF SV40 NON-VIRION PROTEINS

Livingston, D. M., Sidney Farber Cancer Center Inc., Sidney Farber Cancer Institute, *44 Binney St., Boston, Massachusetts*, 02115, United States of America

The summary for this project is under entry no. 179.

233. CONTROL OF TUMOR VIRUS GENE EXPRESSION

Cole, C. N., Yale University, School of Medicine, Dept. of Human Genetics, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

The summary for this project is under entry no. 146.

234. CENTRIOLAR ANTIGENS AND INVOLVEMENT IN CELL PROLIFERATION

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The summary for this project is under entry no. 160.

235. BIOLOGICAL PROPERTIES OF SV40 EARLY PROTEINS

Butel, J. S., Noonan, C. A., Baylor College of Medicine, School of Medicine, Dept. of Virology & Epidemiology, *1200 Moursund Ave., Houston, Texas*, 77025, United States of America

The summary for this project is under entry no. 152.

236. BIOCHEMICAL ASPECTS OF VIRAL CARCINOGENESIS

Kit, S., Dubbs, D. R., Trkula, D., Otsuka, H., Qavi, H., Baylor College of Medicine, School of Medicine, Dept. of Virology & Epidemiology, *1200 Moursund Ave., Houston, Texas*, 77025, United States of America

The summary for this project is under entry no. 13.

237. NOVEL METHODS TO MAP VIRAL FUNCTIONS

Sambrook, J., Stow, N. D., Cold Spring Harbor Lab. of Quantitative Biology, *P.O. Box 100, Cold Spring Harbor, New York*, 11724, United States of America

The aim of the work is to use cDNA synthesized from adenoviral and SV40 mRNA in a variety of transformation and marker rescue experiments. In this way it should be possible to assign mutations to specific genes and to determine which viral genes are essential for transformation.

238. REGULATION OF DIFFERENTIATION IN HEPATOCYTES IN VITRO

Isom, H. C., Pennsylvania State University, Milton S. Hershey Medical Center, School of Medicine, Dept. of Microbiology, *500 University Dr., Hershey, Pennsylvania*, 17033, United States of America

The summary for this project is under entry no. 49.

239. MOLECULAR BIOLOGY AND GENETICS OF TUMOR VIRUSES

Temin, H. M., Mertz, J. E., Risser, R. G., Sugden, B., University of Wisconsin, Madison Campus, Medical School, Dept. of Oncology, *307 N. Charles St., Madison, Wisconsin*, 53706, United States of America

The summary for this project is under entry no. 70.

240. GROWTH CONTROL AND VIRAL GENE EXPRESSION

Gurney, E. T., Utah Higher Education System, University of Utah, School of Science, Dept. of Biology, *1400 E. 2nd S., Salt Lake City, Utah*, 84112, United States of America

CREG: Malignancy induced by small DNA viruses (adenoviruses or papovaviruses), DCCP-17, April 1, 1976.

The goal of the proposed research is to clarify the role of the viral genome in transformation by the small DNA tumor virus, simian virus 40 (SV40). The viral genome and viral gene expression will be studied in phenotypically normal revertants derived from an SV40 transformed mouse cell line, SVT2. The revertant cell lines contain integrated SV40 DNA but exhibit a homeostatic pattern of G1 arrest in confluence culture and are not tumorigenic. The two growth properties distinguish these revertant lines from SVT2 and also from other revertant lines previously derived from SV40 transformed mouse cells. The revertants display the nuclear SV40 large T antigen in a growth-dependent manner; rapidly growing cells have detectable T antigen whereas quiescent cells do not. Hybrid cells producing monoclonal antibodies against SV40 T antigens have been isolated to analyze the virus induced proteins present in the revertant cells.

The proposed research seeks to answer a series of specific questions about the viral genome in the revertants. Is the SV40 DNA or its site of integration altered in the revertants? Is there a specific region of the viral genome which is expressed in transformed SVT2 cells but not in the revertants? Is a specific region of the viral genome expressed in the revertants when the cells are growing but not when quiescent? If there is a portion of the SV40 genome which is not expressed, is this controlled at the level of transcription or later? Are any viral gene products altered in amount or physical properties in growing revertant cells? Are any virus-specific proteins altered only in G1 inhibited revertant cells? These questions will be investigated using the techniques of immunoprecipitation, gel electrophoresis, affinity chromatography, restriction endonuclease cleavage, and nucleic acid hybridization.

241. ATTEMPTED ISOLATION CHARACTERIZATION OF UP-PROMOTER MUTANT OF SV40

Livingston, D. M., Sidney Farber Cancer Center Inc., *44 Binney St., Boston, Massachusetts*, 02115, United States of America

This is part of a broader project. A summary of this subproject is not available.

242. CELL SURFACE PROTEINS OF POLYOMA VIRUS AND SV40 TRANSFORMED CELLS (HAMSTERS)

Walter, G. F., Salk Inst. for Biological Studies, *10010 N. Torry Pines Blvd., P.O. Box 1809, San Diego, California*, 92112, United States of America

This is part of a broader project. A summary of this subproject is not available.

243. VIRAL GENETIC INFORMATION IN SV40 TRANSFORMED CELLS (HUMAN, MONKEYS, MICE)

Lebowitz, P., Yale University, School of Medicine, Dept. of Human Genetics, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

This is part of a broader project. A summary of this subproject is not available.

244. REGULATION OF VIRAL FUNCTIONS IN CELLS TRANSFORMED BY POLYOMA OR SV40

Basilico, C., New York University, School of Medicine, Dept. of Pathology, *550 1st Ave., New York, New York*, 10016, United States of America

This is part of a broader project. A summary of this subproject is not available.

B. POLYOMA VIRUS

1. STRUCTURE AND GENOMIC ORGANIZATION

245. ISOLATION OF DELETIONS IN THE NON-CODING SEQUENCE OF THE POLYOMA VIRUS GENOME PRECEEDING THE LATE REGION

Tyndall, C., Imperial Cancer Research Fund, Transcription Laboratory, *Lincolns Inn Fields, London WC2A 3PX, England, United Kingdom*

The heterogeneous capped 5'-termini of the leader sequences spliced onto the bodies of the three late mRNAs of polyoma virus mRNAs all map in the vicinity of 67 map units (Publication No. 66; Reports 63 and 64). In the case of all known eukaryotic mRNAs which have unique capped termini, a sequence homologous to TATAAATA (the so-called 'Hogness box') occurs in the DNA beginning 31 nucleotides before the nucleotide determining the capped end of the messenger. This sequence is commonly thought to be the eukaryotic equivalent of the 'Pribnow box', TATPyATG, which is characteristic of E. coli promotor sequences. The polyoma virus late region does not have an appropriate 'Hogness box' sequence in the expected position, although the related oligonucleotide TAATTAAA does occur some 40-50 nucleotides before the sequences that determine mRNA ends. Similarly, two other examples of transcription units characterized by mRNAs with non-unique capped termini (AD2 DNA binding protein, Baker et al., Cell, 1979, 18, 569; SV40 late proteins) lack homologous AT-rich sequences at the appropriate position. This suggests that the primary function of the 'Hogness box' sequence is the precise positioning of the transcriptional initiation or processing event that determines the 5'-end of an mRNA. To investigate the significance of DNA sequences preceeding those that determine the capped 5'-ends of polyoma virus late mRNAs, we have attempted to construct deletion mutations in this region. Restriction endonuclease Pvu II cleaves viral DNA at four sites: 67.4, 70.0, 92.1, and 8.9 m.u. The smallest Pvu II fragment, 67.4-70.0 m.u., includes the TAATTAAA sequence. Polyoma virus DNA was partially cleaved with Pvu II to yield four circular permuted molecules. Short terminal sequences were removed from these molecules by controlled degradation with T4 DNA polymerase followed by S1 nuclease digestion. Mouse cells were transfected with the modified DNA in the presence or absence of helper tsA DNA. Thus far, no viable or complementable deletions of the Pvu II site at 67.4 m.u. have been found, although viable mutants lacking the sites at 70.0 and 92.1 m.u. were obtained at high frequency. These deletions are in themselves of considerable interest and are being studied further. (Text Abridged)

246. THE INTEGRATION AND EXCISION OF DNA TUMOR VIRUSES

Hassell, J. A., McGill University, Dept. of Microbiology & Immunology, *3775 University St., Montreal, Quebec*, H3A 2B4, Canada

OBJECTIVE: To study the arrangement and expression of polyoma viral sequences in transformed cells in order to understand how acquisition of viral genetic information causes oncogenic conversion of mammalian cells.

APPROACH: As a first step to realizing our objective, we have begun to physically map the polyoma oncogenes and to determine their products. This is being accomplished by elucidating the structure of integrated viral DNA in cells transformed by polyoma virus, by measuring the capacity of cloned, subgenomic fragments of the viral genome to transform cells in culture, and by studying the expression of polyoma genes in transformed cells.

PROGRESS: Results obtained using the approach outlined above have led us to conclude that only half of the early region of polyoma virus is required to initiate and maintain transformation. Moreover, only the small and middle tumor antigens (T-antigens), but not the large T-antigen, are invariably found in our transformed cell lines. These results imply that small and/or middle T-antigen are required to maintain the transformed state.

247. NUCLEOTIDE SEQUENCING OF POLYOMA DNA

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These proposed studies involve the determination of the nucleotide sequence of polyoma DNA. A portion of the sequence in the regions encoding the transformation related functions of hr-t and T-antigen and the region of the origin of DNA replication have already been determined. We propose to continue these sequencing studies by direct Maxam-Gilbert sequencing as well as primed synthesis sequencing by the methods of Sanger.

248. EFFECTS OF HR-T MUTATIONS ON POLYOMA GENE EXPRESSION

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The research in this proposal is a molecular biological approach to understanding the mechanisms of gene expression in the early region of polyoma virus. Efforts revolve around hr-t mutants of polyoma virus, which map in the proximal early region of the viral genome and define a function essential for both virus growth and malignant transformation of cells. Deletion and point hr-t mutants will be analyzed at three levels: 1) DNA sequencing to define the alterations in the viral DNAs, 2) determination of patterns of transcription and RNA splicing of wild type and mutant viral mRNAs, and 3) in vitro translation of viral mRNAs. It is hoped that these experiments will shed some light on the complexities of gene expression operating in this region of the viral genome and possibly also on the questions of the signals or sequences in RNA transcripts essential for proper splicing. Efforts will also be made to isolate temperature-sensitive mutants in the hr-t gene.

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249. THE HR-T MUTANTS OF POLYOMA VIRUS

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This is part of a broader project. A summary of this subproject is not available.

2. VIRUS REPLICATION

250. POLYOMA VIRUS TRANSCRIPTION AT EARLY TIMES AFTER INFECTION

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We have studied the appearance early after polyoma virus infection of nuclear RNA complementary to the E strand of viral DNA, the processing of this RNA and the subsequent appearance of the early mRNAs in the cytoplasm.

Nuclear and cytoplasmic RNAs were isolated from mouse 3T6 cells at different times early in lytic infection by polyoma virus. These RNAs were then annealed to the E and L strands of 32P-labelled viral DNA prepared in vitro by nick-translation. E strand transcription is detectable nine hours after infection and appears to commence before, or initially to exceed, L strand transcription. At this stage the E strand viral RNA per cell is equivalent to no more than five copies of the E DNA strand. L strand transcription rapidly overtakes E strand transcription by approximately twelve hours after infection. At this time there are approximately 20-40 viral RNA molecules per cell. E strand sequences also appear in the cytoplasm, nine hours after infection before detectable L strand sequences. L strand sequences first appear in the cytoplasm twelve hours after infection just before viral DNA synthesis becomes detectable. Thus RNAs complementary to both strands are found in the nuclei and cytoplasm of the cells before viral DNA synthesis commences. Some of the initial E and L strand viral RNA detected in the nuclei of the cells were longer than full length transcripts of the viral genome.

251. INITIATION AND TERMINATION OF TRANSCRIPTION OF POLYOMA VIRUS DNA

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OBJECTIVE: The definition of sites of initiation and termination of transcription of the L strand of polyoma virus DNA.

APPROACH: One approach to this problem is to analyze the frequency with which different segments of this strand are transcribed. Sequences immediately downstream from an initiation site are transcribed more frequently than those immediately upstream. Conversely, sequences downstream from a termination site are transcribed less frequently.

To carry out this analysis, I propose first to clone certain fragments of polyoma DNA in fd phage. This allows the rapid preparation of large amounts of pure single-stranded DNA of specific DNA fragments. These DNAs will be used as hybridization probes to analyze nascent chains of viral RNA labeled only in their growing 3' ends. RNA will be labeled either in vivo by very short pulses of radioactive precursors, or in vitro by incubation of viral transcription complexes with radioactive precursors. Levels of transcription of different regions of the L strand will be compared. By this means, the locations of termination sites and the quantitative importance of transcriptional termination can be ascertained. These results should help to confirm or alter presently available models of viral transcription.

252. TRANSCRIPTION IN POLYOMA-TRANSFORMED CELLS

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We are studying the nature of the transcripts of polyoma-specific RNA in transformed cells to gain some insight into how expression of the viral genome is controlled. We propose to characterize the virus-specific RNA by molecular hybridization, and by cloning and sequencing reverse transcripts in bacterial plasmids. We will also study the topology of the integrated viral genomes by restriction enzyme digestion and hybridization.

253. CHARACTERIZATION OF THE MRNAS CODING FOR POLYOMA VIRUS TUMOR ANTIGENS PRESENT IN PRODUCTIVELY INFECTED AND TRANSFORMED CELLS

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RNA extracted from productively infected 3T6 cells was enriched for Py sequences by hybridization to Py DNA covalently attached to cellulose. Polyadenylated RNA bound to Py DNA was fractionated on denaturing sucrose gradients and fractions were used to prime cell-free protein synthesis in a cell-free system derived from L-cells. mRNA activities encoding the three major early proteins, large (L-T), middle (M-T) and small (S-t). T-Antigens were located following immunoprecipitation of the cell-free products using Pyanti-tumor serum. The results show that mRNA encoding L-T is smaller (about 20.5S) than mRNAs encoding M-T and S-t (about 21:5S) which are not separated by this procedure. These findings are consistent with the current understanding of early mRNA structure, as determined by S1 mapping (see Report 65) and of its functions deduced from the recently proposed model for Py early gene expression (see Report 87). In this model, all three early proteins are encoded by spliced mRNA molecules. M-T and S-t mRNA are of similar sizes (due to the similar sizes of proposed spliced regions) and L-T mRNA is smaller (due to a more extensive spliced region).

This technique has also been used to size the early viral mRNAs expressed in a rat cell line transformed by Py (82-rat, see Report 82). The single insert of viral DNA in the chromosome of these cells has been mapped by restriction enzyme analysis and the structures of the early viral RNA molecules have been determined by S1 mapping (see Reports 78 and 65, respectively). The results of this size analysis were used to correlate protein coding functions with RNA structure determined by S1 mapping analysis and thus to deduce which regions of DNA in the integrated viral genome are expressed as protein. For example, a T-antigen-related protein of 7,000 daltons is encoded by an RNA molecule sedimenting at 18-19S and is transcribed from the left hand early region of the integrated viral genome in 82-rat cells.

It is hoped that this approach will be used to study early gene expression in other Py transformed cell lines containing integrated viral genomes that have been mapped.

254. IN VITRO TRANSLATION OF EARLY MRNA FROM CELLS INFECTED WITH POLYOMA VIRUS AND ITS MUTANTS

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Our long-term aim is to understand the interaction of viral and host genes involved in neoplastic transformation. The papovaviruses provide a good model system for such studies since these viruses cause oncogenic transformation through the introduction of a small number of viral genes into the host cell, and such genes can be identified and manipulated rather easily. This proposal is aimed at understanding gene expression of the early region of polyoma virus at the molecular level. Our efforts will be focused on the hr-t region of the viral genome which is essential for cellular transformation in vitro and tumor induction in vivo.

More specifically, we are interested in studying the synthesis of the proteins coded by the early region of the viral genome, which can be immunoprecipitated from infected cells with anti-tumor sera. Cell-free translation of early viral mRNA will provide a means of establishing the viral origin of such early proteins. Moreover, by hybrid-arrest translation experiments, we will determine which viral mRNA species codes for which polypeptide, and map the early proteins on the viral genome.

255. PRECISE LOCALIZATION OF THE CAPPED 5'-ENDS OF POLYOMA VIRUS MRNAS

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The experiments described in Report 63 have been continued to characterize further the capped 5'-ends of late viral mRNAs and to position those of early mRNAs. Polyadenylated RNA from cells infected with the tsA mutant was chemically decapped and labelled at the 5' end with polynucleotide kinase. Viral mRNAs were selected by hybridization to polyoma DNA immobilized on cellulose. These were then rehybridized to plasmid DNAs containing different viral restriction fragments, also immobilized in cellulose, followed by digestion with ribonuclease T1 at high ionic strength. The ribonuclease-resistant 5'-terminal mRNA fragments were eluted and fractionated by electrophoresis on thin urea-polyacrylamide gels. The chain lengths of the various short 5'-labelled RNA fragments were accurately measured. The 5'-terminal 2'-O-methylated nucleotide of each fragment was determined, and in some cases the RNAs were sequenced by the partial enzymatic hydrolysis procedure. These data allow us to position the capped 5'-ends with respect to the known viral DNA sequence (see Report 95).

The late mRNAs begin at positions corresponding to A's at 5098, 5093, 5091, 5084, and 5079 and G's at 5099, 5094, 5077 and 5075. Three of these are the ends mapped previously (see Report 63). The heterogeneous 5'-ends thus occur at nearly every possible purine within a 25-nucleotide-long region. The early mRNAs overproduced by the tsA mutant have two alternative 5'-ends, corresponding to a G at position 148 and an A at 153, within the sequence CTTCCCG*CCTCA*TTTC, where the asterisks indicate capping sites. These sites are 29 and 34 nucleotides after the beginning of a sequence homologous to the 'Hogness box,' TATAATT. The DNA sequence around the capping sites is also highly homologous to that found for many other viral and host mRNAs (C.F. Baker et al., Cell, 1979, 18, 569).

256. THE 5'-ENDS OF EARLY MRNAS PRODUCED BY VIABLE MUTANTS WHICH DELETE THE BEGINNING OF THE POLYOMA VIRUS EARLY TRANSCRIPTION UNIT

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Two curious viable deletion mutants were recently isolated by M. Bendig and W. Folk (University of Michigan, Ann Arbor). dl17 deletes nucleotides 71-159 at the beginning of the early region, while dl75 deletes nucleotides 94-171. Both mutations remove the 'Hogness box' and the sequences which normally determine the capped 5'-ends of early mRNA (see Report 64). The dl75 deletion ends only one base before the ATG initiation codon probably used in all early mRNAs. We have mapped the mRNAs produced by both mutants using one and two-dimensional S1 gel mapping. The 5'-ends were accurately mapped by S1-gel analysis using short 5'-32P-labelled restriction fragments. We find that both mutations result in highly heterogeneous 5'-termini. Some of the new 5'-ends lie before the beginning of the deletion, while others lie after the deletion, in fact within the coding region of the DNA. The amount of early RNA produced by dl17 is normal. dl75 over-produces early RNA. This may be the result of the underproduction of large T-protein (Bendig and Folk, unpublished data) because large T protein negatively regulates early gene transcription. The underproduction may be attributed to the reduced translatability of the dl75 mRNAs. We interpret these results to mean that the 'Hogness box' sequence specifies accurate initiation or capping at precise points, but is not required for efficient transcription. This interpretation is consistent with the heterogeneity of the 5'-ends of late mRNAs (see Reports 63 and 64).

257. LOCALIZATION OF THREE MAJOR CAPPED 5'-ENDS OF POLYOMA VIRUS LATE MRNAS WITHIN A SINGLE TETRANUCLEOTIDE SEQUENCE IN THE VIRAL GENOME

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The nucleotide sequences at the 5'-ends of polyoma virus late mRNAs were determined by direct RNA sequencing of decapped and 5'-32P-labelled ribonuclease T1 oligoribonucleotides. Virus-specific polyadenylated cytoplasmic RNA, isolated from mouse cells late during productive infection, was enzymatically or chemically treated to remove specifically the cap structure (m7Gppp). The unblocked 5'-ends of the viral mRNAs were then labelled enzymatically with 32P, the RNAs were digested with RNAse T1 and fingerprinted. Three oligonucleotides derived from capped termini were isolated and their sequences determined to be pGmACAUUUUCUAUUUUAAGp, p(m) AmCAUUUUCUAUUUUAAGp, and p(m)AmUUUUCUAUUUUAAGp. These oligonucleotides comprise a staggered set 15, 17 and 18 nucleotides in length which share a common 3' sequence for 15 nucleotides. The sequences correspond exactly to the polyoma virus DNA sequences from 66.79-66.46 map units, or between 75 and 92 nucleotides preceding the ATG initiation codon for the capsid protein VP2. Previous results showed that the sequence between 13 and 64 nucleotides preceding the VP2 initiation codon corresponds to oligonucleotides reiterated in the 'leader sequence' which is spliced onto the bodies of the three functionally distinct late mRNAs (Publication No. 150).

The three capped oligonucleotides we have sequenced are derived from the first large predicted T1 oligonucleotide 5' to those detected in the leader sequence. The occurrence of a cap at each purine of a single tetranucleotide sequence reflects microheterogeneity in either transcriptional initiation or in a processing cleaving involved in cap synthesis.

258. MATURATION OF THE LATE MESSENGER RNAS OF POLYOMA VIRUS

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The three mRNAs that encode the capsid proteins of polyoma virus are synthesized from the same region of the DNA and share the same 3' terminal sequence adjacent to the poly(A). They owe their diversity to the occurrence of alternative splicing processes during maturation of these transcripts. Despite their structural similarities the three viral mRNAs are synthesized in different amounts such that the ratio of mVP2:mVP3:mVP1 in the cytoplasm of infected cells is 1:2:6. It is possible that each mRNAS is synthesized in nuclei by a separate splicing mechanism. Alternatively the largest mVP2 may be synthesized initially whilst mVP1 and mVP3 arise by subsequent splicing of mVP2 within the nucleus or cytoplasm.

Our results show that approximately four percent of pulsed or continuously labelled nuclear viral RNAs become cytoplasmic transcripts. More than eighty percent of transcripts of the body regions of these mRNAs on the genome are broken down during processing. However, all mRNA sequences adjacent to poly(A) are conserved and almost quantitatively exported to the cytoplasm. All three late polyoma mRNAs have long half-lives and the larger species are not converted to the smaller species in normal cells or enucleate cytoplasms. It follows from this that the mRNA are synthesized in different amounts in the nucleus

of the infected cell and that the cytoplasmic mRNA ratio reflects differential nuclear synthesis of the three mRNAs. The three polyoma late mRNAs also differ in the internally methylated sequences. These methylations are added early in transcript maturation and the methylation differences may direct processing towards a particular mRNA species early in maturation.

259. CHARACTERIZATION OF THE NUCLEAR PRECURSORS OF LATE POLYOMA VIRUS MRNAS

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The structure of the three polyoma virus late mRNAs is complex. Each consists of an untranslated 5' 'leader' segment spliced to a translated 3' 'body' segment. The leader segment consists of repeats of a short sequence transcribed from between 67 and 66 map units (m.u.) on the viral genome. It has been proposed that these repeats arise from the splicing together of the several copies of the sequence present in a giant, tandemly repeated nuclear transcript of the entire circular viral genome (Publication No. 150, also ICRF Scientific Report 1978 - report 66). In order to gain insight into the biogenesis of these mRNAs, virus-specific nuclear RNA has been studied using the S1 nuclease-gel electrophoresis technique.

Previous work demonstrated the existence of giant, head-to-tail, tandemly repeated transcripts of the entire viral genome. A majority of these transcripts have 5'-ends indistinguishable from those of the mature mRNAs. A significant number of molecules, however, have 5'-ends mapping in the anti-early sequences, at 93 m.u. Non-polyadenylated nRNA molecules have 3' ends either at non-specific points or at 93 m.u. Polyadenylated molecules possess a polyadenylated 3' end at 25 m.u., an identical position to that of the 3' end of the mature mRNAs.

To minimize problems of background signal, and to concentrate on species likely to represent precursors to the mRNAs, rigorously polyA-selected nRNA was investigated using two-dimensional gels. Apart from molecules identical to the mRNA, this nRNA contains tandemly repeated transcripts of the entire genome, with 5' ends at 67 m.u. and 3' ends at 25 m.u. A very small number of these molecules lack the sequences between 66 m.u. and 49 m.u. (that are spliced out in mVP1) next to the polyA tract. Most nRNA molecules possessing splices have a 5' end at 93 m.u. and a 3' end at 25 m.u. and lack the sequences between 66 m.u. and either 49 m.u. or 59 m.u. (the splices found in mVP1 and mVP3, respectively). Unspliced transcripts of this region are also found, in similar amounts.

Analysis of the nRNA present at various times after the addition of the transcription inhibitor, Actinomycin D, to infected cells allowed investigation of the stability of the mRNA. All species were shown to be unstable, with half-lives of about 15 minutes. (Text Abridged)

260. MAPPING OF THE SITES OF INITIATION OF POLYOMA VIRUS-SPECIFIC RNA SYNTHESIS

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OBJECTIVE: To map the sites of initiation of late polyoma virus-specific RNA synthesis.

APPROACH: Nuclear RNA from polyoma virus-infected mouse cells is specifically labeled at its 5' di- or triphosphate termini by incubation with a vaccinia virus 'capping' enzyme preparation. Labeled RNA is hybridized to various restriction endonuclease fragments of polyoma DNA bound to nitrocellulose filters, so that it might be determined to which DNA sequences nascent 5' termini are complementary. By this means the site(s) on the viral DNA at which initiation of transcription takes place can be mapped.

261. STRUCTURAL STUDIES ON POLYOMA VIRUS TUMOR ANTIGENS

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Three tumor antigens (T-antigens) are expressed early during productive infection by Polyoma Virus (Py). Recently, a model for early viral genome expression based on DNA sequence, RNA mapping and peptide fingerprinting data obtained by other groups working at I.C.R.F. has been proposed. We have tested the prediction that all three T-antigens have the same amino terminal (N-terminal) amino acid sequences.

Earlier work in this laboratory using microsequence analysis of the two SV40 T-antigens has shown that these two proteins share N-terminal sequences. This technique, however, is expensive and time consuming and we have utilized a more simple and rapid technique, using fingerprint analysis to identify the N-terminal tryptic peptide of the Py T-antigens.

One of the two shared methionine-containing tryptic peptides present in fingerprints of Py large (L-T), middle (M-T) and small (S-t) T-antigens is resistant to aminopeptidase digestion, whereas all other peptides are degraded. We assume that this peptide is uniquely protected from enzymatic digestion by the presence of a modifying group on the variation-NH2 group. If this is the case then the resistant peptide must be the N-terminal tryptic peptide of the Py T-antigens.

The DNA sequence encoding the common N-terminal region predicts that the N-terminal tryptic peptide of the Py T-antigens is Met.Asp.Arg. Digestion of the Py T-antigens with an arginine-specific enzyme from submaxillary gland releases the putative N-terminal peptide. These data, together with the finding that the peptide has a mobility similar to, but not identical with, the N-terminal tryptic peptide of SV40 T-antigens, strongly suggest that Py T-antigens begin N-acetyl.Met.Asp.Arg.

The finding that the three Py T-antigens share N-terminal amino acid sequences and consequently that M-T does not have a signal sequence raises questions about the mechanism whereby M-T becomes associated with the plasma membrane.

262. MECHANISM OF THE MITOGENIC ACTIVITY OF SV40 AND POLYOMA VIRUS TUMOR ANTIGENS

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The summary for this project is under entry no. 161.

263. CONTROL OF GENE EXPRESSION IN TUMOR VIRUSES AND CELLS

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The summary for this project is under entry no. 141.

264. REGULATION OF POLYOMA VIRUS-DNA AND -RNA SYNTHESIS STUDIED BY THE CONSTRUCTION AND ANALYSES OF VIRAL MUTANTS

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OBJECTIVE: To study how polyoma virus regulates RNA and DNA synthesis during vegetative growth and transformation.

APPROACH: The study is carried out by construction and analyses of mutations in regulatory elements and early genes of the virus.

PROGRESS: Recently I described a type of interaction between polyoma virus and mouse cells that resembles both productive infection and transformation; transformed cells carried approximately a thousand free copies of viral DNA. This DNA has a duplication of the origin of replication. The duplication results in a pronounced increase in the rate of viral DNA replication after infection of permissive cells, and probably explains why replication occurs in semi-permissive cells. We also isolated deletion mutants of a novel class. These mutants are capable of vegetative growth, but deficient in transformation.

265. HOST FUNCTIONS RELATED TO TUMOR VIRUS INFECTION

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Cell mutants defective in DNA replication and/or other functions involved in cell growth have been isolated. A class is temperature-sensitive (ts) for semi-conservative cell and polyoma DNA replication. Two, ts-2 and ts-20, are being studied in detail in an effort to determine the biochemical basis for the ts defect.

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the regulation of interferon production: characterization by intraspecific hybrids. Somatic Cell Genetics 4:677-698, 1978. Dooley and Ozer. Ordered replication of DNA sequences: Synthesis of mouse satellite and adjacent main band sequences. J. Cell. Physiol. 98:515-526, 1979.

266. BIOSYNTHESIS IN NORMAL AND VIRUS-TRANSFORMED CELLS

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The summary for this project is under entry no. 155.

267. REPLICON CONTROL IN NORMAL AND TRANSFORMING CELLS

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The summary for this project is under entry no. 181.

268. STUDIES IN POLYOMA TRANSFORMED CELLS - VIRION PROTEINS

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The purpose of this proposal is to further investigate the proteins which are found in the polyoma virion and to determine their respective role(s) in viral infection, virion assembly and cell transformation. The unique aspects of our proposed investigation include: I. The location and affinity of the various polypeptides associated with the DNA and capsid shell will be determined by in vitro disassembly and reassembly of virions. Optimum physiological conditions will be determined for the EGTA-DTT (chelation of virion associated Ca(plus plus)) dissociation of virions. Dissociation products (core complex and capsomeres) will be biophysically characterized, and protein composition, post-translational modification(s) and Ca(plus plus)) binding site(s) determined. These virion dissociation products will then be used for the in vitro assembly of polyoma virions. II. Identification and isolation of the virion hemagglutinin protein. III. Determination of what effects (i.e., regulatory) the isolated polypeptides and capsomers might have on cellular and viral macromolecular synthesis and on possible involvement in cell transformation. IV. Investigation of the mechanism(s) of intracellular uncoating and the possible involvement of virion polypeptides and histones in the expression of early and late viral functions. V. Temperature sensitive polyoma mutants from appropriate complementation groups will be used to study the virion structure, virion uncoating, virion assembly and regulatory functions of the virion polypeptides.

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269. ASSEMBLY OF VIRUSES, MEMBRANES, AND TISSUE

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The objective of our studies on virus and membrane structures is to analyze the assembly and stability of macromolecular aggregates which exemplify the processes of normal and pathological growth and differentiation. The organization of the nucleohistone in polyoma virus is being studied by X-ray cyrstallographic analysis of virus and capsid crystals. The stability and hydration of several small viruses are being characterized by X-ray scattering measurements from solutions. Structural transitions in the spirally wrapped myelin membrane array are being investigated by X-ray diffraction and electron microscopy. The structural basis of electrical and chemical connections between cells is being studied by X-ray diffraction and electron microscopy of purified gap junctions from mouse heart and liver. New X-ray diffraction techniques are being developed to explore the molecular dynamics and structure of viruses and membrane assemblies.

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3. VIRUS-HOST CELL INTERACTION

270. STUDIES ON THE MECHANISM OF ACTION OF TUMOR VIRUSES

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The long-range objective is to understand the biochemical mechanisms by which DNA-chromatin of the virus and the host cell interact to effect viral oncogenesis. To this end studies have been initiated which use mouse cells temperature-sensitive (ts) in DNA-chromatin replication as a biochemical-genetic probe of the host cell contribution to viral DNA replication and the integration of virus DNA into host genome; a prerequisite to neoplastic transformation. We have established that ts A1S9 and ts C1 mouse L-cells are ts in an S-phase function, and that ts 2 cells are ts in a G1 function. Because polyoma (Py) virus functions normally in temperature-inactivated ts A1S9 cells, these can be used to advantage to study Py minichromosome replication and the virus-induced cell DNA synthesis, using both in vivo and in vitro systems. With ts C1 and ts 2 cells which do not support Py formation at the non-permissive temperature (npt) we should be able to identify at least two cellular proteins which are required for Py DNA replication.

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271. DEVELOPMENT OF A EUKARYOTIC CLONING VEHICLE

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Clones of mouse fibroblasts that contain hybrid DNA molecules composed of sequences from polyoma virus DNA, the prokaryotic plasmid pBR322 and the yeast leu2 gene will be examined. These cells should survive in leucine-free medium because the recombinant DNA contributes genetic information for an enzyme involved in leucine biosynthesis. This functional gene provides the basis for the biological selection of cells containing recombinant DNA.

The studies proposed here will define the potential of this polyoma-leu2-pBR322 hybrid DNA as a eukaryotic cloning vehicle. The number of copies per cell, and whether recombinant DNA has integrated into the host cell DNA or replicates autonomously, will be established. A physical map of restriction enzyme sites on one isolate will be constructed and used to identify sites at which additional sequences may be inserted into future experiments. The number of cells producing polyoma early (T) antigen will be determined by indirect immunofluorescence. The cold labile yeast enzyme beta-isopropylmalate (beta-IPM) dehydrogenase can be identified and quantitated in cell extracts by photometrically assaying the production of alpha-ketoisocaproic acid from beta-IPM. Experiments will be conducted to determine optimum conditions for growth of recombinant DNA-containing cells, and for transfection of mouse fibroblasts and other eukaryotic cells.

272. CONTROL OF HISTONE SYNTHESIS BY DNA TUMOR VIRUSES

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The summary for this project is under entry no. 192.

4. CELLULAR TRANSFORMATION

273. MECHANISM OF CELL TRANSFORMATION BY POLYOMA VIRUS

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Hr-t mutants of polyoma virus are used as biochemical tools in a three part investigation into the mechanism of cell transformation by this virus. The first part deals with genetic manipulations on the virus itself. Hr-t mutants appear to be defective in both middle-sized (56K) and small (22K) T antigens. To better understand the role of each of these proteins in the hr-t mutant phenotype, we shall attempt to isolate and study mutants with a normal 22K protein but abnormal 56K protein. The second part deals with the role of the hr-t gene in inducing chromatin modifications of both viral and cellular chromatin. The third part is an investigation of the possible roles of the various polyoma T antigens as protein kinases, cyclic-AMP binding proteins, or substrates for cellular protein kinases.

BIBLIOGRAPHIC REFERENCES: Brian S. Schaffhausen, Jon E. Silver and Thomas L. Benjamin. Tumor Antigen(s) in Cells Productively Infected by Wild-Type Polyoma Virus and Mutant NG-18. Proc. Nat. Acad. Sci. USA 75, 79-83, 1978. Jon E. Silver, Brian S. Schaffhausen and Thomas L. Benjamin. Tumor Antigens Induced by Nontransforming Mutants of Polyoma Virus. Cell 15, 485-496, 1978.

274. EARLY GENES OF POLYOMA VIRUS AND THE ESTABLISHMENT AND MAINTENANCE OF CELLULAR TRANSFORMATION

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OBJECTIVE: To identify the early genes of polyoma virus and assess how they operate in the establishment and maintenance of transformation.

APPROACH: Transformation of permissive and non-permissive cells by early ts mutants of the virus, as well as fragments of wild-type or mutant viral DNA cloned in E. coli. Characterization of the viral tumor antigens expressed in the transformants. Selection of phenotypic revertants and study of the expression of viral genome in these cells.

PROGRESS: Permissive transformants containing the whole viral genome have been obtained using the early ts mutant ts-P155. Non-permissive transformants were selected after exposure to recombinant DNA containing only part of the early region of polyma virus DNA (that included in fragment Hind III-A).

275. ANALYSIS OF THE POLYOMA GENOME IN TRANSFORMED CELLS

Folk, W. R., Rothwell, V., Hart, R., University of Michigan, Ann Arbor Campus, Medical School, Dept. of Biological Chemistry, *1335 E. Catherine St., Ann Arbor, Michigan*, 48104, United States of America

The maintenance of polyoma virus in transformed syrian hamster cells is being studied. The site of integration of thermosensitive A gene mutants is being characterized by Southern blot hybridization; the expression of 'early' gene products of the resident viral genome is being characterized by immunoprecipitation of 35S methionine-labeled proteins; a non-selective system that does not require expression of the 'transformed phenotype' will be used to study maintenance and segregation of polyoma DNAs.

BIBLIOGRAPHIC REFERENCES: Berkner, K.L., and Folk, W.R. (1979) A Map of the Sites in the Polyoma Genome Cleaved by Endonuclease AlyI, Virology, in press. Berkner, K.L., and Folk, W.R. (1979) Quantitation of the Various Termini Generated by Type II Restriction Endonucleases Using the Polynucleotide Kinase Exchange Reaction, J. Biol. Chem., in press.

276. THE ROLE OF TWO POLYOMA EARLY GENES IN TRANSFORMATION

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The objective of this project is to gain some understanding of the role of two early functions of polyoma virus in transformation: the hr-t function and the ts-a function. Complementation between the two classes of transformation defective mutants representing the two functions governs different steps of transformation. This project deals with the analysis of the properties of transformed clones obtained by complementation, referred to as complementation clones. Of particular interest is the question of which viral genes are required to be persistently expressed in complementation clones, and what their relative topology in the host cell is. The method involves the analysis of rescued virus, of the viral integration and of the proteins produced in the transformed cells.

277. CELLULAR CHANGES ACCOMPANYING NEOPLASTIC TRANSFORMATION OF POLYOMA VIRUS

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The hr-t gene of polyoma virus is known to play a major role in inducing changes in cell structure and behavior that accompany malignant transformation. Changes in the cytoskeleton (loss of stress fibers) and cell surface (lectin agglutinability), as well as induction of growth in soft agar or as a focus, are all controlled in some manner by the hr-t gene. This proposal aims at investigating further the cytoskeletal changes which accompany morphological transformation using an immunofluorescent approach with anti-cytoskeletal protein antibodies. Effects of 'local anesthetics' (tetracaine, dibucaine) chlorpromazine and calcium on this process will also be studied.

New efforts will be made toward establishing an in vitro cell system for transformation of epithelial cells by polyoma virus. Attempts will be made to characterize the cellular changes accompanying such transformations in terms of cytoskeletal, membrane and growth properties.

278. POLYOMA VIRUS TRANSFORMATION OF MAMMALIAN CELLS

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Polyoma virus functions expressed by the early region of the viral genome will be studied. Altered viruses lacking restriction endonuclease sites in the early region, as well as revertants of existing thermosensitive mutants, have been isolated. They, together with naturally occurring polyoma virus variants, will be characterized with respect to the type of mutational alteration, and to the effects of the alteration upon the biological properties of these viruses. The ability of these viruses to a) replicate DNA, b) stimulate host cell DNA synthesis, and c) induce cell transformation (abortive and stable) will be analyzed. The products of the viral early region (RNAs and proteins) will be characterized.

279. STUDIES IN POLYOMA TRANSFORMED CELLS - VIRION PROTEINS

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The summary for this project is under entry no. 268.

280. POLYOMA GENE FUNCTIONS AND THE TRANSFORMED STATE

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This is part of a broader project. A summary of this subproject is not available.

281. CELL SURFACE PROTEINS OF POLYOMA VIRUS AND SV40 TRANSFORMED CELLS (HAMSTERS)

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The summary for this project is under entry no. 242.

282. REGULATION OF VIRAL FUNCTIONS IN CELLS TRANSFORMED BY POLYOMA OR SV40

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The summary for this project is under entry no. 244.

283. EXPRESSION AND MODULATION OF THE SYNTHESIS OF ESTERASES DURING IN VITRO DIFFERENTIATION OF TRANSFORMED CELLS (ABBREV)

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The summary for this project is under entry no. 214.

284. REGULATION OF DNA SYNTHESIS IN VIRUS-TRANSFORMED CELLS

Cuzin, F., Clertant, P., Gaudray, P., Perbal, B., Mougneau, E., RassoulzaDegan, M., Universite de Nice, Centre de Biochimie, *Parc Valrose, 06034 Nice, France*

The summary for this project is under entry no. 213.

285. CELLULAR AND VIRAL CONTROL OF ONCOGENIC TRANSFORMATION

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The summary for this project is under entry no. 210.

286. BIOSYNTHESIS IN NORMAL AND VIRUS-TRANSFORMED CELLS

Basilico, C., Carroll, R., Defendi, V., Rush, M., Szer, W., New York University, School of Medicine, Dept. of Pathology, *550 1st Ave., New York, New York*, 10016, United States of America

The summary for this project is under entry no. 155.

287. MAMMALIAN CELL TRANSFORMATION BY ONCOGENIC VIRUSES

Folk, W. R., Vollmer, P., Deans, R., Rothwell, V., Hart, R., University of Michigan, Ann Arbor Campus, Medical School, Dept. of Biological Chemistry, *1335 E. Catherine St., Ann Arbor, Michigan*, 48104, United States of America

Polyoma viruses with nucleotide alterations at specific sites in the early region are being constructed. Numerous mutants with deletions near the origin of DNA replication have been isolated and partially characterized. Some of these are altered in their ability to replicate DNA, or to express gene products. These mutants are being sequenced. Other mutants with alterations in the coding sequences for middle and large T-antigen, or for large T-antigen alone, have been isolated and partially characterized. The biological properties of these viruses are under study.

BIBLIOGRAPHIC REFERENCES: Berkner, K. L., and Folk, W. R. (1979) A Map of the Sites in the Polyoma Genome Cleaved by Endonuclease Alu 1, Virology 92, 482-494. Bendig, M. M., Folk, W. R., and Gibson, W. (1979) Polyoma Viruses with Mutations at Endonuclease Hind II site 1; Alterations at the COOH Terminus of VP 1, J. Virol. 30, 515-522.

288. REGULATION OF POLYOMA VIRUS-DNA AND -RNA SYNTHESIS STUDIED BY THE CONSTRUCTION AND ANALYSES OF VIRAL MUTANTS

Magnusson, G., Karolinska Institutet, Dept. of Biochemistry, *Solnavagen 1, S10401 Stockholm 60, Sweden*

The summary for this project is under entry no. 264.

289. ISOLATING POLYOMA TEMPERATURE-SENSITIVE HR-T MUTANTS

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The major goal of this proposal is to elucidate the role of the hr-t region of the polyoma genome which is essential for neoplastic transformation. Point mutants within the hr-t region with a temperature sensitive phenotype will be sought using several approaches in which mutagenesis is directed to the hr-t region of the viral DNA.

Mutants with the desired temperature-sensitive phenotype will be screened and selected using both productive infection and transformation assays. If temperature-sensitive hr-t mutants can be isolated, they will be characterized genetically and physiologically to confirm their genotype. Of particular interest will be temperature shift studies to directly assess the role of the hr-t function in the maintenance of transformation and to further delineate the difference between the ts-a and hr-t functions in the transformation process.

290. VIRAL GENE FUNCTIONS AND REGULATION OF CELL GROWTH

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Our objective is to clarify the role of polyoma T antigens in cell transformation. We will isolate mutants affecting the medium T antigen of polyoma. We will construct recombinant DNAs to allow the T antigens to be synthesized in bacteria, or in high amounts in mammalian cells. We will study functions associated with the T antigens, particularly a protein kinase activity of unusual specificity in polyoma T antigen immunoprecipitates. We will seek to relate T antigen functions to mechanisms of cell growth control by studying the control of transcription in polyoma infected and transformed cells, and early viral functions such as mitogenic activity and events leading to anchorage-independent growth.

BIBLIOGRAPHIC REFERENCES: Hutchinson, M.A., Hunter, T. and Eckhart, W. Characterization of T-antigens in polyoma-infected and transformed cells. Cell, 15, 65-77 (1978). Hunter, T., Hutchinson, M.A. and Eckhart, W. Translation of polyoma virus T antigens in vitro. Proc. Nat. Acad. Sci. USA, 75, 5917-5921 (1978).

291. PROTEIN KINASE ACTIVITY ASSOCIATED WITH POLYOMA VIRUS MIDDLE-T ANTIGEN

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In an attempt to ascribe biological functions to the tumor antigens of polyoma virus (Py), we have asked whether these proteins have an associated protein kinase activity, similar to that associated with the src gene product of the RNA tumor viruses. Using the assay described by Collett and Erikson (Proceedings of the National Academy of Science, USA, 1978, 75, 2021) and using antiserum raised against Py-transformed cells (anti-T serum) we can detect a protein kinase activity in immunoprecipitates of extracts of Py-infected cells. The activity is not present in uninfected cells, nor is it detected using control serum. Using anti-T serum raised in rats, the proteins phosphorylated in vitro by the kinase are Py middle-T and rat IgG heavy chain; using hamster anti-T serum only middle-T is phosphorylated.

Experiments using a number of mutants in the early region of Py DNA show that the kinase activity is associated with Py middle-T. Thus the amount of kinase activity detected is under the control of the viral A gene but the activity itself is not temperature-sensitive when isolated from tsA-infected cells. The activity is affected by mutations in the hr-t region and in the region shared by large-T and middle-T. The kinase activity, like middle-t, is present in purified preparations of plasma membranes.

The kinase is also detected in Py-transformed cells but not in untransformed cells nor cells transformed by other viruses. Some of the Py-transformed cells that contain kinase activity lack full-sized Py large-T but all contain middle-T. The activity is not detected in a cell line (18.37) which contains integrated viral DNA of a non-transforming hr-t mutant, and which contains Py large-T but not middle-T nor small-t. Together all these results show that Py middle-T or a protein which specifically binds to it has protein kinase activity in vitro, and they raise the possiblility that protein kinases play an essential role in papovavirus-induced transformation.

So far, attempts to demonstrate phosphorylation of middle-T in vivo have been ambiguous. Structural studies show that the phosphate residue attached to Py middle-T in vitro is present in the carboxy terminal half of the molecule, but we have not yet shown unequivocally that similar modification occurs in vivo. (Text Abridged.)

292. MACROMOLECULAR SYNTHESIS AND CELL GROWTH CONTROL

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OBJECTIVES: Firstly, to understand the way in which polyoma virus causes cellular transformation. To do this we will investigate the functions and subcellular locations of the three T antigens of polyoma virus - the 90,000, 60,000 and 22,000 dalton products of the early region of polyoma virus. By the use of monospecific sera and partial purification we hope to be able to define the roles of the individual T antigens.

Secondly, to investigate the mechanism by which the 60,000 dalton product of the Rous sarcoma virus sar gene (p60src) effects cellular transformation. p60src is a protein kinase with the unusual specificity of phosphorylating tyrosine. Our efforts will concentrate on identifying the intracellular targets of this protein kinase. This task will be aided by using the criterion that any substrate of p60src should contain the rare modified amino acid, phosphotyrosine.

BIBLIOGRAPHIC REFERENCES: Hunter, T., M.A. Hutchinson, W. Eckhart, T. Friendmann, A. Esty, P. LaPorte and P. Deininger. Regions of the polyoma genome coding for T antigens. Nucl. Acid Res., 7:2275-2288 (1979). Sefton, B.M., T. Hunter and K. Beemon. Temperature-sensitive transformation by Rous sarcoma virus and temperature-sensitivie protein kinase activity. J. Virol., 33:220-229 (1980).

293. CONTINUED STUDIES OF ISOANTIGENIC AND OTHER GENETICALLY DETERMINED VARIATIONS IN SOMATIC CELLS

Klein, G., Klein, E., Karolinska Institutet, Medical Nobel Inst., Dept. of Tumor Biology, *S10401 Stockholm 60, Sweden*

Our work on the Burkitt/EBV system and a variety of experimental systems has led to the conclusion that viral oncogenesis is a complex process where viral transformation (immortalization) is only the first step. Additional cytogenetic changes, often observed as specific chromosomal anomalies are required before in vivo tumorigenicity is obtained. Somatic hybrid studies involved human and murine lines. They were explored with regard to a variety of genetic markers, and also differentiation related, viral and tumor associated marker. Detailed patterns are complex and cannot be summarized in this space, but have been described in a series of publications. New variant lines have been developed for continued genetic studies, including a battery of 'universal fusers'. Antigenically deficient and/or immunoresistant sublines have been selected from high-antigenic tumors by repeated passage of antibody coated cells on immunoglobulin columns. The immunoresistant variants have a reduced antibody binding capacity. They have permitted a first study of the question of whether serologically defined surface antigen markers also serve as rejection targets. A new polyoma virus induced membrane antigen system was detected on polyoma transformed tumor cells. Its relationship to T-antigen components is being explored in a collaborative study with Ito (London).

C. OTHER PAPOVAVIRUSES

294. SEARCH FOR BK VIRUS SPECIFIC SEQUENCES IN THE DNA OF HUMAN TUMORS

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This project was suggested by the knowledge on BK virus collected in recent years: 1) BK virus is a human papovavirus producing a widespread infection in normal human population; 2) it transforms in vitro cells of hamster, mouse, rabbit and monkey origin; 3) it is highly oncogenic in vivo in hamsters and mice.

We therefore decided to test the possibility that BK virus could be related to human tumors. All the most common tumors of man, but especially tumors of the central nervous system and the urinary tract, will be analyzed since BK virus induces tumors of the central nervous system in experimental animals and is isolated from urine in humans.

Two techniques will be used: 1) DNA/DNA reassociation kinetics; 2) the 'blotting' technique of Southern where tumor cell DNA is digested with restriction endonucleases, separated in agarose gels, transferred to nitrocellulose paper and directly hybridized to a highly radioactive probe of BK virus DNA.

295. HUMAN PAPOVAVIRUS BKV - GENETICS, BIOCHEMISTRY AND RELATIONSHIP TO SV40

Manaker, R. A., Lai, C., Dhar, R., Khoury, G., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Virus Tumor Biology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The genomic positions for the early and late mRNAs of BK virus, a human papovavirus, were determined. The 5' ends of the two early mRNAs produced in lytically infected human embryonic kidney cells, and in BKV transformed human cells, are located at approximately the 0.66 map unit; their 3' ends are at the 0.16 map unit. Intervening genomic sequences deleted from one early species lie between 0.585 and 0.53 map units and those deleted from the other, between 0.535 and 0.53 map units. The genomic body sequences for the 16S species of late BKV RNA are positioned between 0.93 and 0.16 map units. Sequences for the 19S species of late RNA are between 0.765 and 0.16 map units. A leader sequence transcribed from 0.70 to 0.76 units of the viral genome was detected. The structure of late BKV RNAs synthesized in African green monkey cells appeared to be identical to that from human embryonic kidney cells. A comparison of these data with those for SV40 shows both viruses to be closely related.

BIBLIOGRAPHIC REFERENCES: Manaker, R.A., Khoury, G. and Lai, C.-J.: The spliced structure of BK virus mRNAs in lytically infected and transformed cells. Virology, 1979, in press.

296. DNA SEQUENCE OF CONTROL REGIONS OF BK VIRUS (BKV)

Dhar, R., Khoury, G., Seif, I., Lai, C., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Virus Tumor Biology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The purpose of this project is to obtain a nucleotide sequence of the regulatory region of the BKV genome. A comparison between different papovaviruses may help us correlate gene structure to function. The nucleotide sequence of the late region, coding for the proteins VP1, VP2 and VP3 has been obtained. The part of the genome which codes for the large T-antigen has been obtained. A rapid technique was developed to determine the sequence of more than two thirds of the genome.

BIBLIOGRAPHIC REFERENCES: Dhar, R., Seif, I. and Khoury, G.: Nucleotide sequence of the BKV DNA segment encoding small t-antigen. Proc. Natl. Acad. Sci., U.S.A. 76: 565-569, 1979. Seif, I., Khoury, G. and Dhar, R.: BKV splice sequences based on analysis of preferred donor and acceptor sites. Nucl. Acids Res., 1979, in press.

III. ADENOVIRUS

A. STRUCTURE AND GENOMIC ORGANIZATION

297. STRUCTURE AND FUNCTION OF THE ADENOVIRUS GENOME

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The long-range objective of the research program is to elucidate the organization and function of the genome of the human adenovirus, type 5. This knowledge is necessary in order to understand the host-viral interactions that lead to an alteration of the regulation of cellular growth associated with cell transformation. Specifically, I propose to isolate and characterize deletion mutants of the virus which will allow the identification and mapping of viral genes and the study of the physiological function of the gene products. Included amongst these mutants will be those that are transformation defective and are therefore altered in genes that are necessary for the transformation process. These genes can be identified and the gene products characterized.

298. STRUCTURE OF GENES OF ADENOVIRUSES INVOLVED IN CELL TRANSFORMATION

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OBJECTIVE: Recently investigators in our laboratory were able to localize the genes of several adenoviruses (non-oncogenic as well as oncogenic) involved in cell transformation at the left terminus of the respective viral DNA molecules. By determining the entire nucleotide sequence of this region in a few subtypes of this virus it is hoped to contribute to an understanding of the organization of the transforming genes.

APPROACH: Viral DNA fragments which display transforming activity are mapped and sequenced by procedures that are now standard in nucleic acid biochemistry. The primary structure of the transforming DNA is then collated with knowledge about the location and size of viral messenger RNA and of the protein products encoded by the transforming genes.

PROGRESS: a) The left terminal 8% and a considerable portion of the leftmost 16% of adenovirus 5 DNA have been sequenced. This sequence when viewed in the light of recent information about mRNA and transforming proteins has given an overall picture of the structure of the transforming genes of adenovirus 5. b) The left terminal 8% (which also contains the transforming genes) of the weakly oncogenic adenovirus 7 has been sequenced.

BIBLIOGRAPHIC REFERENCES: J. Maat and H. van Ormondt, accepted for publication in Gene (1979). A. de Waard et al. Proc. XI Int. Cong. Biochemistry, Toronto 1979.

299. THE PHYSICAL ORGANIZATION OF THE ADENOVIRUS GENOME AND THE CONTROL OF VIRAL GENE EXPRESSION IN INFECTED CELLS

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We are continuing to construct a map of the expression of the human adenovirus 2 genome by using mRNA purified by hybridization to fragments of viral DNA and by size separation, followed by cell-free translation, to identify the polypeptides encoded by viral mRNAs. Precise location on the Ad2 DNA sequence of the coding region for each protein will be accomplished by peptide mapping and partial amino acid sequence analysis of viral proteins synthesized in vitro. To date, we have identified 33 viral polypeptides whose genes have been at least approximately mapped. Of these, the amino terminal sequences of EIB-15K, IX, II, and IV have been determined to exactly locate the coding sequences for these polypeptides.

It is now clear that the viral cycle is more complicated than implied by the conventional division into an early and a late phase. To study the regulation of expression of viral mRNAs, we are determining which mRNAs are present in cells infected with various mutant viruses, and in cells treated with diverse inhibitors of proteins and of DNA synthesis.

300. ORGANIZATION AND REGULATION OF THE ADENOVIRUS GENOME

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The long-range goal of the research program is to elucidate the nature of DNA tumor virus gene products, the manner in which the expression of these gene products is regulated, and their effect on host cell regulation. Specifically, I propose to isolate adenovirus deletion mutants in two very different ways. The first approach is to select for pre-existing deletion mutations in Ad 5 DNA preparations on the basis of a reduction in the number of restriction endonuclease cleavage sites. The second approach will be to induce the formation of deletions in the adenovirus genome using acridine dyes. In each case the mutations will be located on the adenovirus physical map and their physiological consequences will be determined.

301. PROPERTIES OF ADENOVIRUS DNA

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The major objective of these studies has been the application of physical and biochemical techniques to map and to define the structure of specific genetic regions in the genomes of both oncogenic and nononcogenic human adenoviruses, with the ultimate goal of (1) relating these sequences to those biochemical activities which permit these viruses to influence or gain control of cellular functions (e.g., cell lysis and transformation) and (2) of defining basic mechanisms for regulation of genetic expression (e.g., initiation of DNA synthesis). A number of unique features have been described which may have implications in viral replication or carcinogenesis or both.

302. TRANSFORMING DNA SEQUENCES OF ADENOVIRUSES

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OBJECTIVES: Characterization of adenovirus transforming DNA sequences.

APPROACH: Hybridization and reassociation techniques are being used to characterize transforming DNA sequences present and viral specific mRNA synthesized in cells transformed by the transforming DNA segment.

PROGRESS: The transforming gene(s) of adenovirus type 12 and type 7 were found to be located at the end of the molecule. The 3Y1 cell line, a clonal cell line from Fischer rat embryo, could be transformed by the Ad12 EcoRI-C (left hand 16%), the Ad12 HindIII-G (left hand 7%) or the Ad7 HindIII-I.J (left hand 8%), and several cell lines tranformed by these transforming segments were established. Integrations and transcriptions of viral genome fragments in these cell lines are being investigated in detail using recently developed procedures such as Southern's hybridization, spot hybridization, and nuclease S1 analysis with conventional hybridization and reassociation kinetics methods.

303. DNA SEQUENCE ANALYSIS ON HUMAN ADENOVIRUS GENOMES

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The proposed work involves the structural studies and sequence analysis of biologically important regions of three members of human adenovirus DNAs from serotypes 2, 7 and 12 which are respectively non-, weakly or highly oncogenic serotypes. First the inverted terminally repetitious region, a unique structural feature, commonly shared by all the serotypes so far tested, will be sequenced. This region is probably biologically important because it has been shown to be a portion of the transforming segment of the DNA which has been characterized in other laboratories in at least three adenovirus serotypes, 2, 7 and 12. The purpose of this work is also to find a correlation, if any, between the length of this region and its nucleotide sequence homology to the oncogenic potential of some important adenovirus serotypes. During the course of this work in our laboratory, some interesting aspects of the structural uniqueness in adenovirus serotype 2 were discovered. A protein of MW 55,000 daltons is covalently attached at or near the 5' ends of adenovirus 2 DNA. Further studies on the biological significance of the tight binding of a protein at the two ends of Ad 2 DNA are in progress in view of the fact that the origins of DNA replication have been located near the two termini.

304. DNA SEQUENCE AND COMPUTER ANALYSIS OF A TUMOR VIRUS

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Recent improvements in the technology available for the determination of nucleic acid sequences have made it feasible to sequence the entire adenovirus type 2 (Ad2) genome. However, the quality of sequence data and the speed at which this data accumulates make it no longer practical to assemble or analyze this information without computer assistance. Programs will be

developed to assist in both of these functions. The programs developed will be of general application; considerable emphasis will be placed on the ability of the computer to recognize patterns and generate hypotheses which can be tested experimentally. Specific programs will be written to: (1) Assemble DNA sequences from raw data; (2) search a completed sequence for translational start points, reading frames, stop points and restriction enzyme recognition sites and hence to predict certain aspects of the biochemical properties of DNA sequences; (3) assist in the determination of unknown restriction enzyme recognition sites; (4) search a DNA sequence such as the oncogenic or transforming region of Ad2 for other features which may be of biological interest. In addition, the feasibility of automating the reading of autoradiographs containing sequence data will be explored.

305. FUNCTION OF DNA VIRUS GENOMES IN ANIMAL CELLS

Carter, B. J., de la Maza, L. M., Laughlin, C. A., Myers, M. W., Jay, F. T., Marcus, C. J., Moss, B., Doerfler, W., Westphal, H., Ward, D., Shenk, T., Jones, N., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Arthritis Metabolism & Digestive Diseases, Chemical Pathology Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

We are employing DNA viruses as molecular probes to study genome expression in human cells. We are studying intensively the structure and function of adeno-associated virus (AAV) since this virus has only a single gene. We are studying also adenovirus which is a more complex genome. Adenovirus is the helper virus for AAV multiplication. This helper relationship is being analyzed. In addition, both AAV and adenovirus recombine with cellular DNA. In the case of adenovirus this causes malignant transformation of the cell. AAV can inhibit this transformation and also inhibits Ad12 oncogenesis in newborn animals. Thus AAV inhibits tumor induction. The mechanism of this inhibition of tumor induction is being studied at the molecular level in both cell culture and in animal experiments.

BIBLIOGRAPHIC REFERENCES: B. J. Carter, C. A. Laughlin, L. M. de la Maza and M. W. Myers: Adeno-associated virus autointerference. Virology 92: 449-462, 1979. C. A. Laughlin, M. W. Myers, D. L. Risin and B. J. Carter: Defective-interfering particles of the human parvovirus adeno-associated virus. Virology 94: 162-174, 1979.

306. THE EFFECT OF DELETIONS AT THE 3' END OF THE SV40 EARLY REGION ON THE ADENOVIRUS HELPER FUNCTION

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The summary for this project is under entry no. 151.

307. MAPPING OF ADENOVIRUS 2 PROTEIN CODING SEQUENCES

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The objective of this project is to map the early and late protein coding sequences on the adenovirus 2 genome. In addition, experiments will be conducted to determine if the removal of the 5' leader sequences of late messages or genes has any effect upon in vitro translation or linked transcription-translation. Both the translation and linked transcription-translation systems were developed by the principal investigator. Should time permit, a study of the reasons for the inhibition of host protein synthesis in late infection will be undertaken.

The methods to be employed in solving these problems include hybridization and in vitro translation. For example, restriction endonuclease DNA fragments will be hybridized to mRNA isolated from KB or HeLa cells during early or late infection. Hybrid arrested translation (HART) will then be used to map protein coding sequences relative to restriction endonuclease cleavage sites on the adenovirus genome. HART relies upon the fact that mRNA hybridized with its complementary DNA sequences is not translatable into protein in a cell-free system. Heat melting of this hybrid restores the ability of the mRNA to direct protein synthesis. The hybridization of specific restriction endonuclease fragments with populations of mRNAs allows one to correlate the appearance (heat dissociation of hybrids) and disappearance (unmelted hybrids) of specific protein bands with specific DNA fragments. The protein can thus be mapped to its structural gene sequence. HART has already been used to map the late protein coding sequences with respect to the Bam I cleavage sites.

308. THE GENERATION AND CHARACTERIZATION OF MUTATIONS IN THE EARLY REGION 4 AND AT THE ORIGINS OF REPLICATION OF ADENOVIRUS TYPE 5

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The aim of this project is to generate and characterize mutants in the early region 4 and the double, cis acting origin of replication termini of adenovirus type 5. Mutants in the early region 4 will be isolated by the method of Jones and Shenk (1978), and mutagenesis of the adenovirus termini will be attempted by Shortle and Nathans (1978) in vitro mutagenesis.

309. ADENOVIRUS GENOME EXPRESSION - PHYSICAL MAPPING STUDIES

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A major objective of the research is to investigate and exploit the empty viral capsids and incomplete particles of subgroup B human adenoviruses. Emphasis is given to in vitro studies of adenovirus assembly, especially with regard to DNA packaging. One goal is to elucidate mechanisms of sequential protein-DNA interactions which result in the compaction of DNA within the confines of the capsid and which determine the left end polarity of capsid DNA association in vivo. The transfection potential of an effective in vitro system for packaging DNA molecules in the capsid of an animal virus is also of interest. Parallel with this is further understanding of the biological activity of incomplete adenovirus particles in abortive infections. These incomplete particles contain the DNA sequences from the left end transformation-active region of the adenovirus genome.

A second objective in this project is to develop methods for physical mapping and manipulation of viral genomes. Work thus far has centered on studies of branched DNA structures and the process of branch migration leading to strand displacements. Our efforts have recently led to a general method for site-directed deletion mutagenesis in closed circular DNA.

Recombinant DNA technology has recently been pursued, resulting in plasmid-cloned sequences from the entire Ad 3 genome. Our objective is the application of these clones for the study of DNA packaging in vitro as well as for probes in experiments related to viral genome expression and transformation. Using the deletion mutagenesis method, we hope to identify the specific viral DNA sequences which direct entry of DNA into empty adenovirus capsids in vivo and in vitro.

BIBLIOGRAPHIC REFERENCES: Tibbetts, C. and C. Giam (1979). In vitro association of empty adenovirus capsids with double-stranded DNA. J. Virology 32:995-1005. Green, C. and C. Tibbetts (1980). Targeted deletion of sequences from closed circular DNA. Proc. Nat. Acad. Sci. U.S.A., in press.

310. GENE EXPRESSION OF HUMAN ADENOVIRUSES

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This is part of a broader project. A summary of this subproject is not available.

311. MOLECULAR ANALYSIS OF ONCOGENIC VIRUSES

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A program to investigate the structure and properties of both DNA and RNA tumor viruses is proposed. A major component will be the application of recombinant DNA methods to viral systems. For adenovirus these methods can be used to

generate new types of mutants with which to analyze viral physiology. For a variety of retroviruses, including the Friend, Abelson, Moloney, AKR and MCF leukemia viruses as well as the Moloney sarcoma virus, this approach offers the opportunity to learn about the architectural details of their genomes with great precision. It should also allow the application to retroviruses of genetic methods that have previously only been applicable to DNA tumor viruses.

Among the proposed areas of investigation are studies of the details of the mechanism of reverse transcription, the transfectability of transformed phenotypes of chemically transformed cells, the genetic organization and expression of the spleen focus forming virus component of the Friend leukemia virus complex, the genetic structure of endogenous retroviruses of wild mice and the expresson of adenovirus genes.

312. GENETIC ANALYSIS OF ADENOVIRUSES

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This research project aims to define the organization of the adenovirus genome in terms of the location and function of the virus genes which control virus development during infection, and to define the requirements for adeno gene products in oncogenic transformation of rodent cells. Temperature-sensitive (ts) and host-range (hr) mutants of type 5 human adenovirus have been isolated, and these are being characterized genetically and physiologically. Many of these mutations have been genetically mapped, and have now been mapped physically by marker rescue with an improved transfection method using adeno DNA-protein complex. The hr mutations are defective for transformation and map within the left-hand early gene sequences. These have been partially characterized in terms of viral DNA synthesis and early viral mRNA synthesis; the results provide evidence that a very early viral gene product encoded by sequences between 1.5 to 3.5 units regulates expression of other early regions. The hr mutants are now being characterized in terms of early viral protein production, induction of cellular DNA synthesis and induction of transplantation immunity. The mutations of one set of early function, transformation-defective ts mutants (ts 36, ts 69, ts 149) have been mapped between 18.5 to 22.0 units on the genome. Additional early gene sequences have now been located in this region, and these ts mutants are being further characterized genetically and physiologically. The improved transfection method is being used to introduce specific fragments mutated or modified in vitro into the Ad5 genome.

313. ORGANIZATION AND EXPRESSION OF GENES IN VIRAL DNAS

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The summary for this project is under entry no. 109.

314. DNA AND RNA TUMOR VIRUSES - MOLECULAR MECHANISMS OF INFECTION AND CARCINOGENESIS

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The summary for this project is under entry no. 128.

B. VIRUS REPLICATION

315. THE CHARACTERIZATION OF TRANSCRIPTION IN ADENOVIRUS

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Previous studies have shown that transformation by adenovirus-2 requires integration into the host chromosome of only a small fraction of the total genome, the left 7.5%. Two mRNAs, 15s and 11s, are coded by this portion of the genome. Previous analysis of the 15s RNA has shown that it is the first mRNA coded by the extreme left end of the adenovirus-2-genome. The start point for this mRNA has been mapped about 500 nucleotides from the end of the genome. Preliminary examination of RNA present in the nucleus suggests that these 500 nucleotides, or some part of them, are transcribed. It is the purpose of this proposal to accurately map the 5'-terminal sequence of this 15s RNA within the DNA sequence of the left end of the adenovirus-2 genome and to further map the 5'-end of the nuclear transcripts. These transcripts will then be isolated by hybridization to a fragment of adenovirus-2 DNA containing the left 4.4% of the genome. Extracts from the nuclei of infected cells will be prepared and assayed for their ability to cleave these nuclear RNA sequences and produce the correct mRNA sequence.

316. ADENOVIRUS TRANSCRIPTION AND GENE PRODUCTS

Sharp, P. A., Massachusetts Inst. of Technology, Center for Cancer Research, *77 Massachusetts Ave., Cambridge, Massachusetts*, 02139, United States of America

We have continued our investigations of the process of viral gene regulation during human adenovirus infection. In 1977, we discovered that most late adenovirus 2 mRNAs have a tripartite leader segment spliced to their 5' terminus. This tripartite leader set is coded for by DNA sequences mapping at 17, 19, and 26 on the genome. These findings led us to propose that late adenovirus 2 mRNAs synthesis involved the intramolecular processing of long initial transcripts which span viral DNA sequences coding for both the tripartite leader set and the body of the mRNAs. We have characterized the structure of steady state nuclear RNA during the late state of infection, and shown that their structure is consistent with this model. In addition, these data suggested that the splicing was primarily sequential, from 5' to 3'. In collaboration with Jim Manley and Malcolm Gefter in the Department of Biology at M.I.T., we have shown that nuclei prepared from late infected cells can carry out many of the processes involved in late Ad2 mRNA synthesis. In particular, late Ad2 mRNA synthesis is initiated in these nuclei by RNA polymerase II and we have shown that these in vitro synthesized long nuclear RNAs have the expected 3' termini.

Dr. Arnold Berk in my laboratory has developed a procedure to accurately map viral mRNAs on the Ad2 genome. This procedure involves forming RNA/DNA hybrids between a single strand of viral DNA and mRNA and digesting this hybrid with a single strand specific nuclease. The protected DNA fragment is then resolved by electrophoresis in an agarose gel. Using these procedures, we have defined the structures of both early SV40 and Ad2 mRNAs and are in the process of establishing their pathway of synthesis.

317. REGULATION OF TRANSCRIPTION OF ADENOVIRUS GENES BY HUMAN KB CELLULAR RNA POLYMERASES

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The object of this proposal is to study the regulatory mechanisms underlying transcription of human cellular genes using membrane-free, cell-free systems containing human KB cell RNA polymerases and necessary factors. Human adenovirus is chosen as the model system for these studies. Most genes of adenovirus are probably transcribed by RNA polymerase II and two genes by RNA polymerase III. However, it is not clear how the polymerases interact with the template, whether factors other than purified RNA polymerases are required for faithful transcription and what the primary transcripts are. Recently I have successfully developed the first eukaryotic DNA-directed membrane-free, cell-free system from human KB cells for this purpose (Wu, G. J. (1978) Proc. Nat. Acad. Sci. USA, 75: 2175-2179). Faithful transcription of adenovirus specific 5.5S RNA has been demonstrated in this system by the analyses of size, sequence and genomic location of the product. The system opens up the first possibility for studying the molecular mechanism of transcription of eukaryotic genes by human or eukaryotic RNA polymerase in vitro. The present work will focus upon 1) further characterization of 5.5S RNA synthesized in vitro by sequence analysis, 2) fractionation of factors other than human RNA polymerase III required for proper initiation and termination, 3) comparison of the nuclear and the cytoplasmic RNA

polymerase III in terms of their specificity of transcription, and 4) molecular interaction of regulatory factors with human KB RNA polymerase III and the 5.5S RNA gene and determination of the sequence of the promoter site of this gene. After the above studies on RNA polymerase III have progressed considerably, attempts will be directed at devising a cell-free system for faithful transcription of other adenovirus genes by human KB cell RNA polymerase II.

318. ACCURATE TRANSCRIPTION OF ADENOVIRUS IN VITRO

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The aim of the proposed research is to purify and study all factors required for accurate initiation at the adenovirus 2 (Ad2) major late promoter.

We will begin with the in vitro transcription system of Manley, Sharp, and Gefter (unpublished results). This system consists of purified Ad2 DNA and a crude protein fraction. The protein fraction is prepared by lysing HeLa cells in a Dounce homogenizer in a hypotonic buffer, treating the lysed cells with 10% (NH4)2SO4 to lyse the nuclei, centrifuging at 100,000xg and concentrating the protein in the supernatant by (NH4)2SO4 precipitation. Transcription is initiated accurately at the Ad2 major late promoter by RNA polymerase II endogenous to the extracts.

RNA polymerase II will be purified from this extract using accurate initiation, rather than nucleotide incorporation as an assay. All factors required for accurate initiation at the Ad2 major late promoter will also be purified. These factors will be studied, using techniques developed for the study of procaryotic transcriptional regulatory proteins, to attempt to identify a function for each factor.

319. STUDIES ON IN VITRO TRANSCRIPTION OF ADENOVIRUS EARLY GENES

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Recent reports from the laboratory of Dr. Robert G. Roeder have demonstrated the feasibility of establishing faithful in vitro transcription systems using purified, or partially purified, eukaryotic components. For example, specific and accurate initiation of transcription has been observed at the major late promotor of adenovirus 2 using purified viral DNA and RNA polymerase II in the presence of a cell-free extract derived from uninfected cells.

The purpose of the proposed investigation is to attempt similar reconstitution experiments focusing, instead, on the 5 promotors for those genes expressed early in the adenovirus lytic cycle. The immediate goal is to establish in vitro conditions which promote specific transcription from these various early promotors and then to define required activities present in the cell-free extract. The investigation will be conducted from a comparative point of view in order to determine whether accurate initiation can be observed at each of the early promotors in the presence of the simple cell-free extract, or whether some or all of these promotors require additional components. Variables will include the nature of the viral template, as well as the source and fraction of the soluble extract. The long-term goal is to develop a reconstituted transcription system which can then be used to define the role of molecules which may regulate transcription of these genes during the lytic cycle. Finally, it is also hoped that this study can be extended to a study of transcription of the early adenovirus genes in the transformed state.

320. MECHANISMS OF ADENOVIRUS-2 TRANSCRIPTIONAL CONTROL

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We have previously investigated the locations and structures of adenovirus-2 promoters, the arrangement of genetic information and mRNA species within the viral genome, and the basic pathway of processing of viral mRNA. We now propose to investigate the molecular mechanisms which regulate the complex program of RNA transcription and processing used by adenovirus-2 (Ad-2) to express its genome during productive infection. We shall focus attention upon transcriptional control including: 1) The "cascade" activation of viral promoters, in particular the mechanism of the early to late transcription switch; 2) the role of protein:DNA interactions and chromatin conformation in transcriptional control; 3) the establishment of soluble or subcellular Ad-2 transcription systems which retain regulation in vitro. Specifically we propose: To determine whether late transcription is controlled at the RNA initiation step. To determine the role of termination and anti-termination in regulating Ad-2 late transcription. To identify transcriptional control steps for early and intermediate transcription units which appear to be specifically regulated. To assay for specific binding of RNA polymerase II and the Ad-2 72K single stranded DNA binding protein to promoters or DNA sites which regulate viral transcription. To measure changes in viral chromatin structure at promoter, terminator, or other sites, which accompany changes in transcription unit expression. To prepare sub-cellular transcription systems (nuclei, transcription complexes, naked DNA) which retain regulated transcription patterns, and to assay for (bio) chemical conditions or proteins which can bring about the transcription changes normally seen in vivo.

321. REGULATION OF TRANSCRIPTION

Wu, G., Emory University, School of Medicine, Dept. of Microbiology, *1364 Clifton Rd. N.E., Atlanta, Georgia*, 30322, United States of America

The object of this project is to elucidate the regulatory mechanisms of transcription of human cellular genes at the molecular level. Adenovirus is used as the model system for in vitro transcription system for studying regulation of transcription by RNA polymerases II and III plus necessary factors. We will focus on isolation of necessary regulatory factors required for selective and accurate transcription by both enzymes and study their interactions with the proper sequence on DNA template.

BIBLIOGRAPHIC REFERENCES: Wu, Guang-jer (1980), Faithful Transcription of Adenovirus 5.5 S RNA Gene by RNS Polymerase III in a Human KB Cell-Free Extract, J. Biol. Chem. (in press)

322. ADENOVIRUS 2 CODED EARLY GLYCOPROTEIN

Wold, W. S., St. Louis University, School of Medicine, Institute for Molecular Virology, *1402 S. Grand Blvd., St. Louis, Missouri*, 63104, United States of America

An Ad2 early glycopolypeptide, gp25K, has been purified to homogeneity. A partial amino acid sequence of gp25K will be obtained, in order to map its structural gene on the Ad2 genome. The structure of the oligosaccharide moiety on gp25K will be characterized. A monospecific antiserum against gp25K will be used to study the regulation of the gp25K gene. Mutants in and about the gp25K gene will be isolated and studied.

BIBLIOGRAPHIC REFERENCES: Wold, W.S.M. and Green, M. (1979) Adenovirus type 2 early polypeptides immunoprecipitated by antisera to five lines of adenovirus transformed rat cells. J. Virol. 30, 297-310. Brackmann, K.H., Green, M., Wold, W.S.M., Cartas, M., Matsuo, T., and Hashimoto, S. (1980) Identification and peptide mapping of human adenovirus type 2 induced early polypeptides isolated by two-dimensional gel electrophoresis and immunoprecipitation. J. Biol. Chem., in press.

323. EARLY GENE TRANSCRIPTION IN ADENOVIRUS 2 INFECTED CELLS

Zimmer, S. G., University of Kentucky, School of Medicine, Dept. of Pathology, *Limestone & Euclid, Lexington, Kentucky*, 40506, United States of America

The overall objectives for this project are to: 1) Define the nature of the transcriptional, processing, and transport controls that are regulating the expression of viral RNA at early times during a productive infection and 2) conduct studies that provide clues to the functions of the early gene products.

The specific projects involve the role of DNA repair in studies involving transcriptional mapping of UV-inactivated viral genomes. These studies are also being extended to determine the general effects of DNA binding compounds on viral gene expression.

The general nature of transcription of regions not coding for early RNAs is being studied by examining both self-annealed and nonself-annealed early viral nuclear RNAs.

The effects of early viral gene expression on tumorigenicity of transformed cells and the effects on cell surface changes is being monitored through the use of plant lectins and the immunoprecipitation of viral coded proteins.

BIBLIOGRAPHIC REFERENCES: Hurley, Laurence H., Chandler, Coralie, Garner, Timothy F., Petrusek, Ruby, and Zimmer, Stephen G. DNA binding, induction of unscheduled DNA synthesis, and excision of anthramycin from DNA in normal and repair-deficient human fibroblasts. The Journal of Biological Chemistry, 254 (3): 605-608, 1979.

324. ANALYSIS OF ADENOVIRUS GENE TRANSCRIPTION IN VITRO

Luse, D. S., Washington University, School of Medicine, Dept. of Biological Chemistry, *660 S. Euclid Ave., St. Louis, Missouri*, 63110, United States of America

We propose to attempt the development of a system for the faithful in vitro synthesis of adenovirus late mRNA. The experiments will be based on the successful in vitro synthesis, by RNA polymerase III, of adenovirus VA RNA already accomplished in this laboratory. Specificity of transcription will be assayed by localizing initiation to the known major late promotor at 17 map units on the R strand of the adenovirus genome. RNA will be synthesized using purified KB cell RNA polymerase II and templates of increasing complexity (naked adenovirus DNA or restriction fragments thereof; nucleoprotein complexes reconstituted from adenovirus DNA and cellular chromosomal or viral proteins; and isolated, infected KB cell nuclei). These template-RNA polymerase combinations will be supplemented by nuclear or cellular extracts (similar to those successfully employed in the RNA polymerase III/VA RNA system) if this proves necessary to obtain specific transcription.

325. REGULATION OF EXPRESSION OF DNA TUMOR VIRUSES

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Transcription of adenovirus type 2-DNA in a cell-free system is being investigated in order to determine what controls expression of DNA-tumor virus genes. The regulatory system involved in switching from early to late RNA synthesis is being characterized. This is achieved by using nuclear extracts from uninfected early and late infected KB cells to synthesize radioactive protein. After isolation and physical characterization these proteins are tested for their ability to suppress or stimulate RNA synthesis.

326. REGULATION OF GENE EXPRESSION IN DNA TUMOR VIRUSES

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Transcription of adenovirus 2 RNA sequences specified by the early gene region responsible for cell transformation will be studied in both lytically infected and transformed cells. Distinct viral nuclear RNA species will be identified and precisely mapped within the viral genome by hybridization to various restriction endonuclease fragments of viral DNA. Pulse-chase experiments will be done to study the metabolism of these RNAs, and to establish a precursor-product relationship between nuclear viral RNA and cytoplasmic mRNA. A system using isolated nuclei from early-infected cells will be developed to study the metabolism of nuclear viral RNAs; RNAs synthesized in isolated nuclei will be compared with those made in intact cells. The effects of protein synthesis inhibitors (cycloheximide) and of temperature-sensitive mutations in the viral genome on the transcription of these RNA sequences will be investigated in infected and transformed cells.

327. REGULATION OF ADENOVIRUS GENE EXPRESSION

Roeder, R. G., Weil, P. A., Honda, B., Segall, J., Luse, D., Matsui, T., Boguski, M., Ginsburg, A., Washington University, School of Medicine, Dept. of Biological Chemistry, *660 S. Euclid Ave., St. Louis, Missouri*, 63110, United States of America

The objective of this work is to elucidate mechanisms involved in the regulation of viral gene transcription. The first major emphasis is on adenovirus genes which encode the VA RNAs and on cellular genes which encode the 5S and tRNAs. Purified or cloned DNAs containing these genes have been shown to be accurately transcribed by purified (host) class III RNA polymerases in the presence of other factors isolated from uninfected cells. In future studies we will (1) attempt to complete the purification of the factors necessary for the transcription of the purified genes by the purified RNA polymerases; (2) determine whether they are the same for different cellular and viral genes transcribed by RNA polymerase III; (3) determine whether these factors are sufficient to activate the same genes in standard viral nucleoprotein or cellular chromatin structures and, if not, (4) search for other cellular or viral induced transcription factors which act in conjunction with these factors, and which will restore the regulation of these genes observed in vivo; (5) investigate the mechanism(s) of action of the various transcription components in terms of sequence specific binding to DNA, the induction of chromatin structural modifications, etc.

The second major emphasis will be on those viral genes which encode mRNAs and which we have recently shown can also be accurately transcribed by RNA polymerase II and other factors in a reconstituted cell-free system. We will continue to focus on the major late transcription unit, to which our analyses have thus far been restricted, and will investigate this system as outlined for the class III genes. We will concomitantly initiate similar studies of the adenovirus early genes.

BIBLIOGRAPHIC REFERENCES: P.A. Weil, J. Segall, B. Harris, S. Ng, and R.G. Roeder, Faithful Transcription of Eukaryotic Genes by RNA Polymerase III in Systems Reconstituted with Purified DNA Templates, J. Biol. Chem. 254, 6163-6173 (1979). M. Green, R. Lebovitz, and R.G. Roeder, Expression of the Autonomous Parvovirus H1 Genome: Evidence for a Single Transcription Unit and Multiple Spliced Polyadenylated Transcripts, Cell 17, 967-977 (1979).

328. COTRANSCRIPTS OF VIRAL AND HOST DNA IN CELLS PRODUCTIVELY INFECTED OR TRANSFORMED BY AN ONCOGENIC VIRUS

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OBJECTIVE: To investigate the amount, composition and role of cotranscripts of viral and host DNA in cells infected or transformed by an adenovirus.

APPROACH: RNA is extracted from productively infected cells or rat embryo cells transformed by adenovirus type 2 (Ad2), cell-line 8617. Viral RNA is selected by hybridization to Ad2DNA-cellulose. The selected RNA is analyzed by hybridizations to viral or host DNA and by electron microscopy.

PROGRESS: Productively infected KB cells contain few, if any, cotranscripts. The transformed cells, line 8617, do contain cotranscripts but the exact number is not yet known. Procedures for obtaining pure viral RNA have been improved. An electron microscopy technique for analyzing viral RNA molecules has been developed.

329. POSSIBLE ROLE OF PREMATURELY TERMINATED RNA CHAINS IN GENE REGULATION

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This project will explore whether prematurely terminated RNA chains that have been discovered as products from several different adenovirus transcription units and appear to be present from cell transcription units as well may be involved in gene regulation. Techniques of nucleic acid biochemistry and cell biochemistry will be employed to test this probability. Sequences near the 5' site for transcription units of both virus and cell genes have been located and appropriate molecular hybridization studies will be used to detect short promoter proximal chains even when the whole transcription unit is not active.

330. INVESTIGATION OF THE MECHANISM OF MRNA FORMATION USING ADENOVIRUS AS A MODEL

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The overall objective of the project is to elucidate the details of messenger RNA biogenesis in order to build a frame-

work for an understanding of the manner in which gene expression is controlled.

Adenovirus mRNA production will be utilized as a model system since the processes involved in adeno mRNA formation all appear to be analogous to those of the uninfected cell. The availability of large amounts of a well mapped DNA genome and the fact that a large proportion of infected-cell RNA synthesis is viral offer clear advantages to the use of this system.

Two general areas will be investigated: the role of internally methylated residues (m6A) in mRNA formation and a characterization of the nature of premature transcription termination. Specifically, the locations of m6A residues in mRNA, the conservation of m6A residues, and the timing of the addition of m6A residues will be determined, primarily with regards to processing of nuclear RNAs.

Premature transcription termination from the late adeno promoter at map position 16 may be analogous to attenuation in bacteria. Studies on this subject will include a) the development of a quantitative assay for premature termination, b) an analysis of the structure of prematurely terminated transcripts and c) an analysis of the other adenovirus transcription units for premature termination.

331. THE MECHANISM OF REGULATION OF ADENOVIRUS 2 EARLY MRNA PRODUCTION

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The left 11% of adenovirus 2 codes for at least four mRNAs in lytic infection, one of which is not found in adenovirus 2 transformed cells. Additionally, adenovirus 2 is generally believed to use host cell mechanisms for the production of its mRNA. This system allows us to ask how an animal cell can regulate the production of two mRNAs from the same DNA segment. In order to differentiate between transcriptional and post - transcriptional models, it is necessary to decide if one transcriptional unit produces a single primary transcript which is processed to form multiple mRNAS, or if the boundaries of the transcriptional unit change to new primary transcripts.

The only way to decide between these possibilities is to define the boundaries of transcription. To accomplish this I have proposed three experiments which can unambiguously identify the active sites of initiation of transcription in either adenovirus 2 transformed cells or lytically infected cells. If the sites of initiation of adenovirus 2 transformed cells are the same as the sites in lytic cells, where different mRNAs are made from the same DNA segment, then a post-transcriptional model is likely. Alternatively, if the initiation sites change, the transcriptional control is indicated.

332. BIOSYNTHESIS OF ADENOVIRUS EARLY RNAS

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Mutations will be introduced by enzymatic and chemical methods into localized regions at the left end of the adenovirus 5 genome. In separate experiments mutagenesis will be directed to the 5' and 3' ends of the early region encoded there (early region 1, the transforming region of Ad5) and into areas where sequence is joined in spliced mRNAs from this early region. Host range mutants generated by these methods will be selected according to their ability to grow on 293 cells, a human cell line transformed by Ad5 which expresses early region 1 functions, and their inability to grow on HeLa cells. mRNA negative mutants will be isolated by screening the generated host range mutants with the sensitive and simple S1 mapping method to identify mutants which fail to induce early mRNAs in HeLa cells. mRNA negative mutants will be mapped by marker rescue and sequenced. An adaption of the S1 mapping procedure to the analysis of pulse labeled transcripts will be used to characterize the biochemical defect in these mutants. By extensively mutagenizing localized regions throughout early region 1, it should be possible to identify all steps in mRNA production which require specific DNA and RNA sequences and to define these sequences. Determining the sequence of mutations which revert the mRNA negative mutants will yield insight into the mechanisms of these steps.

The host range mutants obtained in these studies will be placed into complementation groups and representatives of each complementation group will be assayed for their ability to transform rat embryo and hamster embryo cells. Such studies will define the Ad5 genes required for transformation by Ad5.

333. METABOLISM OF ADENOVIRUS MESSENGER RNA

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The objective of these studies is to characterize the metabolism of messenger RNA synthesized by oncogenic human adenoviruses during productive infection. These studies will include the analysis of viral RNA synthesized in vivo and in isolated nuclei. In addition to the synthesis of viral RNA, the processing, transport and degradation of viral RNAs will also be investigated. For most of these studies the metabolism of individual viral RNA species will be analyzed; this will be achieved by the use of unique viral DNA fragments as hybridization probes.

334. STUDY OF RNA INITIATION AND PROCESSING IN ADENOVIRUS

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Transcription and RNA processing in eucaryotes must be controlled in part by nucleotide sequences around the startpoint of transcription and around the cleavage and splicing points in the RNA. To identify the functions of some of these regions of DNA utilized in expression of the late genes of adenovirus, I propose to insert fragments of adenovirus-5 DNA into a cloning vehicle. The effects of the insert will be monitored by analyzing RNA products coded by genes downstream from the insert. The cloning vehicle will be an adenovirus-2 - adenovirus-5 derivative with a single cleavage site for a given restriction enzyme, contained within the nonessential region, and deletions amounting to approximately 10% of the genome. The fiber structural gene lies immediately downstream from the nonessential region. Therefore, analysis of the structure of nuclear and cytoplasmic fiber RNA products from cells infected with the recombinant will detect whether the insertions are directing additional transcription and/or processing. The initial insertion will extend from a few hundred base pairs upstream from the putative startpoint of late transcription through two of three segments normally spliced together at the 5' end of mature late mRAs.

335. CHARACTERIZATION OF ADENOVIRUS-2 RNA TRANSCRIPTS BY ELECTRON MICROSCOPY

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Adenovirus-2 RNA transcripts isolated at various times from the nucleus or the cytoplasm of infected cells will be characterized by electron microscopy with respect to their sequence arrangements and pathways of synthesis.

Specific Ad-2 transcripts will be enriched by preparative hybridization to defined restriction fragments of Ad-2 DNA, followed by chromatography on various affinity columns or by size fractionation using sedimentation and gel electrophoresis. These RNAs will be mapped by electron microscopy of hybrids formed with single- or double-stranded DNA. Leader sequences and other inserted or deleted segments in the transcripts can be identified by observation of secondary hybridization to remote segments of the DNA strand or by single-strands of DNA restriction fragments added as third components during annealing. Nuclear RNA will be screened for possible intermediates in processing, and the order in which the segments of the mature RNA are joined should become apparent. I will also examine whether the 3' ends of RNAs are determined by transcription termination or by processing from longer nuclear transcripts. In lieu of identified mutations in the leader region, representatives of related adenovirus classes will be compared with respect to their leader sequences and processing mechanisms. Correlation of the EM conclusions with other biochemical, physiological, and genetic studies of adenovirus transcription should help elucidate the mechanisms resulting in the extraordinary structures

of the mature mRNA species, and may suggest reasons for their existence.

336. METABOLISM OF ADENOVIRUS RNA

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The object of the proposed investigation is to gain additional insight into the molecular events and regulatory implications in messenger-RNA metabolism in cultured mammalian cells. Human (HeLa S3) cells infected by adenovirus and rat embryo cells transformed by adenovirus will be employed in these investigations. In the lytic infection the following aspects of viral RNA metabolism will be explored. Transcription of the genome will be examined by electron microscopy following dispersal of nuclear contents. Processing of apparent large molecular weight nuclear-precursor RNA will be examined by analysis of 5'-termini and by analysis of RNA synthesized in the presence of adenosine analogues. Fate of cytoplasmic viral RNA will depend upon limited RNA structure analysis, especially if viral RNA sequences which remain in the nucleus can be isolated. In addition, information bearing on the mechanism of formation of virions and restriction of host-cell RNA synthesis should also be elucidated. The feasibility of extending these investigations to adeno-2-transformed rat embryo cells, which will depend on the incorporations of sufficient radioactivity, will be constantly evaluated. These investigations should not only bear on the cell biological implications of the biogenesis of messenger-RNA but also on the infection and transformation of cultured mammalian cells.

337. EARLY MRNA SPECIES TRANSCRIBED FROM GENE BLOCK III ON ADENOVIRUS GENOMES (ABBREV)

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Various early mRNA species transcribed from early gene block III (75-86 map units) on the adenovirus-2, Ad2 (plus) ND1 and Ad2 (plus) ND5 genomes will be isolated. The cDNA of these transcripts made by reverse transcription will be cloned into different vectors, i.e., pBR322 and M-13. The recombinant plasmids containing individual mRNA species will be characterized by direct DNA sequence analysis using Sanger's chain termination method. A substantial part of the Ad2 genome and small segments of Ad2 (plus) ND1 and Ad2 (plus) ND5 genomes (75-87 m.u.) have already been sequenced. Direct DNA sequence analysis of the genomes will be continued. Comparison of the mRNA (cDNA) sequences with genomic sequences will help in assessments of the role of primary sequence in the control of expression of gene block III mRNAs, early SV40 mRNAs and host range specificity functions. Study will help identify the early promotor, transcription-initiation and termination signals, various mRNA processing sites, and may give clues to understanding the mechanism of mRNA biogenesis. Computer analysis will be conducted on the primary mRNA and genomic sequences to identify the stable secondary structure in this region of the genome.

338. MOLECULAR BIOLOGY OF ADENOVIRUSES

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OBJECTIVE: By use of adenovirus lytic infection we hope to study synthesis in eukaryotic cells. Of special interest are control mechanisms involved in initation processing and translation of adenovirus mRNA. The expression of adenovirus genes in transformed cells might also function as a model system to study viral transformation and the differences in gene expression between a viral gene in a free and in a transformed state.

APPROACH: We propose to use regular techniques for protein biochemistry, translation in in vivo and in vitro system, and RNA synthesis both in vivo and in vitro. Some specific areas of the genome are beng sequenced and in order to identify many of the late and early gene products we propose cloning cDNA copies of adenovirus messenger RNA in E. coli in collaboration with the group at the Institut Pasteur in Paris. Cloning of some restriction enzyme fragments of the adenovirus DNA is also included in this project.

PROGESS: During the last years we have been able to identify some of the early proteins coded for by the viral genome. Two of them have been purified and an association between a viral encoded glycoprotein and the transplantation antigens has been described. Most of the virus encoded products have been mapped on the viral genome and characterization of the function of non-structural proteins from adenovirus infection is currently in progress.

In vitro system for transcription has been developed and the mechanism of inhibition of a benzimidazole derivative (DRB) is currently investigated. Some messenger RNAs appear to be insensitive to DRB inhibition whereas the majority are inhibited by this drug. Attempts to identify the initiation site and the proteins, if any, involved in the initiation are currently in progress.

Several of the messenger RNAs at early and late times in the infectious cycle have been identified and separated in methylmercuric hydroxide gels. cDNA transcripts are currently being made and cloning of some late messengers has also been achieved in Paris. Future work will focus on the early messenger RNA which might be of importance for the induction and the maintenance of the transformed cell.

339. ORIGIN AND STRUCTURE OF THE LATE ADENOVIRUS 2 MRNA LEADER

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Spliced mRNAs derived from noncontiguous genomic regions have been observed for several eukaryotic genes. In adenovirus 2 a leader sequence originating from three parts of the genome is joined to one of at least eight different late mRNAs. This organization of adenovirus 2 late mRNA raises several important questions about the function and synthesis of the leader, several of which could be answered by sequencing the leader.

I propose, then, to determine the leader sequence for a late mRNA (1OOK-mRNA). 1OOK-mRNA will be purified and will serve as a template for AMV reverse transcriptase. Short restriction endonuclease fragments complementary to the 5'-terminus of 1OOK-specific mRNA will be isolated and used to prime the synthesis of DNA complementary to the entire leader sequence. The rapid DNA sequencing technique developed by Maxam and Gilbert will then be used to determine the cDNA sequence.

I also plan to sequence those nucleotides surrounding each of the three leader components in the adenovirus 2 genome. Restriction endonuclease fragments containing nucleotides both surrounding and including each part of the leader sequence will be isolated and sequenced. These studies should be valuable in assessing potential functions for the leader such as ribosomal interaction and capacity for translation, and also in analyzing late mRNA maturation, in particular the role of leader-neighboring sequences in RNA recombinational events.

340. VIRAL GENE EXPRESSION AND ITS REGULATION IN PRODUCTIVELY INFECTED AND TRANSFORMED CELLS

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The goal of this program is an understanding of the mechanisms by which adenovirus mRNA species are synthesized in lytically infected and transformed cells. As adenoviruses apparently rely completely on their hosts' transcriptional apparatus, this system provides an analogy with eukaryotic gene expression and its regulation that is readily amenable to experimental analysis. The approach to be adopted will combine both in vivo and in vitro experiments. In vivo studies will address such questions as whether or not synthesis of viral mRNA involves processing of larger, nuclear precursors during the early and late phases of productive infection and in transformed cells: putative precursor RNA molecules will be identified both by their size and by the presence of sequences that do not correspond to those present in any mRNA. If precursor mRNA molecules can be identified during any virus/cell interaction, such as in the late phase of productive infection, then, kinetic and sequence relationships between them and specific mRNAs will be defined. These stud-

ies will involve pulse-labelling and pulse-chase experiments with labelled, mercurated ribonucleosides: this approach should permit not only definition of the fate of putative precursors but also their isolation free from other viral RNA sequences and hence their characterization in terms of the regions of the adenovirus 2 genome from which they are transcribed. If it is shown that processing mechanisms do have a role in the biosynthesis of adenovirus mRNA, an appropriate precursor will be isolated and used as a substrate with which to assay for HeLa cell processing enzymes, with the object of purifying and characterizing them.

In parallel to these in vivo studies, in vitro experiments on the transcription of adenovirus 2 DNA and transformed cell chromatin by various RNA polymerases will be performed. The viral RNA sequences transcribed in vitro will be defined and compared to viral mRNA by hybridization to the separated strands of restriction endonuclease fragments of 32P-labelled viral DNA. It is unlikely that naked viral DNA is the template for transcription during productive infection and attempts will therefore be made to define the physical state of this template. (Text Abridged.)

341. CONTROL OF GENE EXPRESSION IN MAMMALIAN CELLS

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The main objective of this research is to gain insights into the mechanisms of control of gene expression in mammalian cells at the biochemical level. We are studying the biosynthesis of adenovirus late mRNA and mRNA precursors in vitro. The system employed supports termination of transcription at the end of structural genes as well as polyadenylation. We will endeavor to analyze these processes in order to understand the biochemistry of mRNA biosynthesis. We are also studying the 'splicing' out of internal sequences of adenovirus late mRNA precursors.

An additional part of this research is the elucidation of the molecular basis underlying all variants of plasmacytoma cells that are not expressing either heavy or light chains of immunoglobulin. The gene organization of the alpha heavy chain is being examined. In addition, the gene organization of tRNA genes is also being examined.

342. THE REGULATION OF ADENOVIRUS GENE EXPRESSION

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Regulation of adenovirus gene expression by the virus-coded 72K early protein is being studied in isolated nuclei. Manipulation of temperature and use of inhibitors of macromolecular synthesis prior to nuclear isolation are used to probe the specificity and mechanism of action of the 72K protein. Conditions that allow de novo initiation of RNA synthesis in vitro will be used to test the effect of the 72K protein on virus transcription, using both cell extracts and purified 72K protein. The action of TPA on adenovirus early transcription will also be investigated in the isolated nuclear system.

Revertants of ts125 will be screened for ability to regulate early virus transcription, and will also be tested for ability to transform rat embryo fibroblasts. The relationship of the 72K protein to cell transformation will be further tested by measurement of transcription rate in vivo in rat embryo cells infected with various mutant alleles of the 72K protein gene.

Investigation of selective procedures for isolating possible Ad5 regulatory mutants is continuing.

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343. ADENOVIRUS EARLY GENE FUNCTION AND DNA REPLICATION

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We propose to study adenovirus DNA replication in vitro with particular regard to the proteins of both cellular and viral origin involved in the process. Specifically we will examine the role of the protein covalently attached to adenovirus DNA in the adenovirus DNA replication process.

Structural studies concerning the adenovirus DNA protein complex are also planned. Specifically, by using a series of proteolytic enzymes, we hope to 'map' the site of DNA linkage within the protein. Attempts will also be made to determine the nature of the linkage between the protein and the DNA. An antibody against the protein will also be prepared.

344. FUNCTIONS OF EARLY ADENOVIRUS PROTEINS IN VIRAL AND CELL DNA REPLICATION AND CELL TRANSFORMATION

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A study of the functions of adenovirus 'early' proteins in DNA replication is proposed. The work will concentrate on the proteins made by the transforming region located at the molecular left end of the viral genome, and is divided into two stages; 1. In vivo: The ability of purified or partially purified proteins from adenovirus-infected cells to complement mutant viruses defective in the transforming region will be tested by microinjection into infected cells. Parallel experiments in which viral mRNA or subgenomic DNA fragments are injected will serve as positive controls and will also allow precise mapping of the defective genes. In addition, the effects of active proteins on DNA synthesis in uninfected cells will be monitored. 2. In vitro: The properties of the purified proteins will be examined in subcellular extracts from cells infected with wild-type or mutant viruses, to identify their site of action in the replication process. The proteins will also be screened for activity in enzymic assays.

These experiments should identify the 'early' proteins which are involved in DNA synthesis and help elucidate the mechanism of virus replication, and may shed some light on the involvement of viral products in the initiation or maintenance of the transformed phenotype.

345. ADENOVIRUS DNA SYNTHESIS AND POLYPEPTIDE ASSEMBLY

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Adenovirus (Ad) DNA replication will be studied in infected nuclear extracts which have been shown to elongate viral DNA by normal intracellular mechanisms. This system has been used to study a temperature sensitive mutant (H5ts125) which is defective in a DNA binding protein (DBP) necessary for Ad DNA synthesis both in whole cells and in nuclear extracts. These DNA replication extracts are dependent on exogenously added DBP. The effects of post-translational modifications such as phosphorylation and polypeptide cleavage of the DBP will be assessed in these complementation assays.

The enzymology of Ad DNA replication can be studied in these in vitro systems. We are purifying DNA polymerases bound to Ad replication complexes and comparing the details of structure and function of these bound polymerases with those that are either found free in the infected cell or in unifected cells. Attempts to reconstitute replication complexes that can synthesize full-size Ad DNA products will be made using purified viral DNAs and various protein fractions. The requirements for specific DNA polymerases as well as other proteins will be examined in the reconstituted replication complexes.

Several selective polymerase inhibitors are available to help compare the requirements for viral DNA replication in whole cells with those in our in vitro systems. Aphidicolin, which we have shown to be an inhibitor of mammalian cell DNA polymerase alpha, will be used. Dideoxythymidine, an inhibitor of gamma polymerase, is also available for these studies. Using this viral model system, we will be able to explore the details of DNA replication in mammalian cells. Some of the differences between viral and cellular pathways may provide clues to the oncogenic potential of the adenoviruses or to ways

that inhibition of adenoviral growth may be achieved by selective inhibition of DNA synthesis.

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346. ADENOVIRUS-INDUCED ALTERATION OF CELL CYCLE EVENTS IN RODENT CELLS

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OBJECTIVE: Human adenoviruses are able to induce DNA replication followed by cytogenetic damage in nonpermissive or semi-permissive rodent cells arrested in the G1 stage of the cell cycle by serum starvation. Our objective is to compare the virus-induced cell cycle with the normal serum-induced cell cycle to obtain information on: a) The mechanism of virus-induced alteration of cell cycle events and controls and b) the significance of altered cell cycle events in transformation and tumorigenesis.

APPROACH: Detailed comparison of virus-induced and serum-induced cycles concentrating mainly on the following: a) Cell cycle kinetics in normal and infected cells; b) virus-induced cell DNA replication-kinetics, order and involvement of viral proteins; c) virus-induced cell-coded enzymes; d) cytogenetic aberrations; e) genetic analysis using virus and cell mutants.

347. REPLICATION MAP OF AN ONCOGENIC VIRUS

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We have developed a rapid, simple, and quantitative filter-binding assay which can separate covalent DNA-protein complexes from protein-free DNA. We have used the assay to detect and quantitate the adenovirus DNA-terminal protein complex. We propose to study the role and synthesis of the terminal protein during infection. We develop a model for the action of terminal protein during initiation of adenovirus DNA replication.

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348. BIOCHEMISTRY OF ANIMAL VIRUS MULTIPLICATION

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Our objectives are to understand the functions and organization of the human adenovius (Ad) early genes. We propose a coordinated investigation of early genes: early proteins (EP), early RNA transcription and processing, Ad DNA replication, and integration of Ad early genes in transformed cells and their expression as nuclear RNA and mRNA. Ad2 EPs will be purified and mapped on the viral genome by partial amino acid sequence and cell-free translation. These studies together with 5'-terminal sequencing of the 13 Ad2 mRNA T1 oligonucleotides and longer sequences mapped in early regions E1-E5 should provide insights into DNA sequences important in transcription initiation, RNA processing, splicing, and translation initiation. Functions of EPs will be studied using in vitro DNA replication and possibly RNA transcription systems, monospecific EP-antisera, enzyme assays, and analysis of the interaction of EPs (and cell proteins) with interesting Ad2 DNA fragments using protection experiments coupled with Maxam-Gilbert sequencing. Transcription studies by Northern blotting and nuclease gel procedures will emphasize E5 and transformed cell transcripts. HE C19 cells, an Ad12 transformed cell line that appears to have an intact late region will be used to investigate regulation. Ad integration will be studied in 5 group C transformed cell lines; Southern blotting studies are completed. We will clone 20 kb pieces of transformed cell DNA and study (1) possible cell and viral sequence specificity at the junction, and (2) possible cell DNA sequences required for Ad integration. Ad DNA replication studies will concentrate on the terminal protein (TP) and the reconstitution of in vitro Ad DNA replication with purified EPs, emphasizing initiation. TP studies will focus on (1) the protein-DNA linkage, (2) genetic origin (antisera, cell free translation, amino acid sequences of group A-E TPs, synthetic oligodeoxynuleotide probes coding TP amino acid sequences), and (3) interaction with Ad DNA, 73K DNA binding protein, and VA RNA, and (4) possible role in DNA replication. Studies on early gene organization and function will be important in illuminating cellular gene transcription and regulation, DNA replication, and growth control.

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349. ADENOVIRUS DNA AND PROTEIN SYNTHESIS (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

350. CARCINOGENS ON PRO- AND EUCARYOTIC DNA REPLICATION

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The control mechanism operating in the in vitro replication of phi XRFI DNA will be continued. The precise role played by the phi X-induced A protein as well as the phi XA(star) protein will be examined.

The further disection of the adenovirus DNA replication system will be continued. This system has now been divided into two major reactions; one involves the elongation of primed Ad DNA and the second involves the initiation of adeno DNA synthesis.

The influence of various carcinogens on each of these DNA replication systems will be carried out.

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351. ENZYMOLOGY OF TUMOR VIRUS DNA REPLICATION

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The enzymology of tumor virus DNA replication is almost completely unknown. An in vitro system of adenovirus DNA replication ('M-system'), which has been developed, will be used to study this problem. The enzymes of DNA metabolism which are found to be present in the system will be characterized and their roles will be studied.

The M-system has been found to be accessible to added protein. In vitro complementation will be used to study the specificity of the activity of the adenovirus-specific DNA binding protein, as well as its role in DNA replication. It will also be used to purify and characterize the product of the other adeno 5 DNA negative gene, represented by the mutant ts36. The use of the M-system will be extended to other animal viruses, such as SV40, herpes and vaccinia, and the complementation method will be applied to DNA negative mutants of these viruses.

The M-system will be used as a starting point for the development of a soluble extract which can support replication of exogenous DNA. Two special problems of virus-cell interaction will also be studied: the permissiveness of cell types for virus infection, and the induction or shut-off of host DNA synthesis during viral infection.

352. INHIBITION OF CELLULAR DNASES BY DNA TUMOR VIRUSES

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The objective of this project is the elucidation of the mechanism of the adenovirus-induced inhibition of cellular DNase which we have observed (Nass, K., and Frenkel, G.D., J. Virol. 26:540, 1978). We have suggested that the viral coded DNA-binding protein may act as a DNase inhibitor, protecting replicating viral DNA molecules from degradation by single-strand DNase. During initial attempts to directly examine the ability of the viral-coded DNA-binding protein to inhibit DNase, we detected a cellular DNA-binding protein which possessed such a DNase inhibitory activity, and which contaminates preparations of the viral protein. We have purified the cellular DNA-binding protein and carried out a study of the mechanism of DNase inhibition. We are preparing antibody to this protein and we will purify the viral DNA-binding protein and determine whether it possesses any DNase-inhibitor activity in the presence of the antibody to the cellullar DNase inhibitor. If it does, then the mechanism of the viral DNase inhibitor will be characterized by the methods used for the cellular protein. We will also study the level of the cellular DNase inhibitor in cells after adenovirus infection, in order to examine the possibility that the virus modulates the levels of DNase in the cell by altering the level of DNase inhibitor.

353. GENETIC STUDIES ON ADENOVIRUS

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OBJECTIVES: The role of DNA-binding virion core proteins in adenovirus infection. Current biophysical and enzymological methods will be used to examine the supramolecular organization and protein composition of the native adenovirus genome in various stages of infection. The problem of whether adenovirus assumes chromatin structure at various points during infection will be particularly studied. The results are expected to clarify the roles core proteins play and are also expected to shed light on the role of cellular enzymes and proteins which may be involved in these processes.

The structure and role of the DNA fragments present in incomplete particles (IP) of adenovirus 2. Ad2-ts4 accumulates IP at 39 degrees and fails to produce mature virus (Khittoo and Weber, 1977). This DNA-encapsidation defective mutant will be used to tackle experiments expected to yield information on: (1) The mechanism of DNA encapsidation, (2) the origin of the DNA present in IP, and (3) the biological mechanisms in viral infection which give rise to IP.

354. GROWTH AND MATURATION OF ADENO-ASSOCIATED SATELLITE VIRUSES

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We will continue to investigate at both cellular and molecular levels, the mechanisms by which adeno-associated satellite viruses (AAV) interfere with the replication and potential oncogenesis of their helper adenoviruses and herpesviruses. We will use conditionally defective helper viruses, namely temperature-sensitive mutants of human adenovirus type 31 and herpes simplex virus, type 1, known to be defective in viral DNA synthesis and/or other virus-specific functions. Complementation studies with these mutants will simplify dissection of the defective satellite replication cycle. AAV are defective at both the replication and maturation steps. We will analyze the products synthesized in these complex systems by the techniques of radioisotope labeling of viral DNA, ultracentrifugal analysis, electron microscopy, column chromatography and polyacrylamide gel electrophoresis, and correlate these parameters with biological function. We will initiate experiments to clone AAV DNA in E. coli in an effort to obtain relatively large quantities of this reagent by a simpler and more economical procedure than that currently available.

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355. INTEGRATION OF AD2 DNA

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This is part of a broader project. A summary of this subproject is not available.

C. VIRUS-HOST CELL INTERACTION

356. INTERACTION OF SV40 AND HUMAN ADENOVIRUSES - A MODEL SYSTEM TO STUDY INTEGRATION AND EXCISION OF VIRAL GENETIC MATERIAL

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The summary for this project is under entry no. 184.

357. VIRAL CARCINOGENESIS AND LATENCY

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Continued study of productive and abortive adenovirus-cell interactions should shed light on regulatory functions that may be crucial to the establishment and maintenance of the malignant state generally. The approaches focus on the modifying control exerted by adenovirus infection on cellular macromolecular synthesis and chromosome duplication. Human adenoviruses are viewed as convenient probes in tumor biology generally rather than as candidates for a major etiological role in naturally occuring human cancer. They are convenient and useful because: (1) They are of human origin. (2) They encompass a spectrum of antigenically, biochemically, and structurally distinct types which differ in their oncogenic capacity. These differences reflect, in turn, the kind of infection a given adenovirus can establish in a given host or in cells derived from the host. (3) A vast amount of detailed knowledge has accumulated about the structure and replication of adenoviruses and, specifically, about information flow from the viral genome to virus-specified transcriptional and translational products. Building on this rapidly evolving background of knowledge about the viruses themselves, we wish further to delineate those regulatory mechanisms operating in the normal cell cycle which are subject to modifications by adenovirus gene expression, or which themselves exert a controlling effect on adenovirus replication.

The experimental systems will involve the use of fresh serum or fractions of plasma and virus infection as activators of cell cycle progress in cells brought to arrest in G1 by various methods, and amino acid deprivation, especially of arginine, as a modifier of both viral replication and cellular macromolecular synthesis.

BIBLIOGRAPHIC REFERENCES: Mannino, R.J., Allebach, E.S., and Strohl, W.A. Encapsulation of high molecular weight DNA in large unilamellar phospholipid vesicles: dependence on the size of the DNA. FEBS Letters 101, 229-232, 1979.

D. CELLULAR TRANSFORMATION

358. MOLECULAR BIOLOGY OF ADENOVIRUSES AND ONCOGENIC TRANSFORMATION

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OBJECTIVE: To define the polypeptides produced from the transforming region of adenovirus serotype 5 and to examine their functions in transforming cells.

APPROACH: To immunoprecipitate viral antigens from radioactively labelled virus-infected cells using tumor antisera and analyse them by gel electrophoresis. To translate, in cell-

free systems, mRNAs purified from virus-infected cells and identify the products. In each case, cells lytically infected and transformed by wild-type and host-range mutant viruses will be studied. A protein kinase activity associated with early viral antigens is being examined.

359. STUDIES ON ADENOVIRUS INDUCED CELL TRANSFORMATION

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OBJECTIVE: The purpose of this project is to understand the mechanisms by which human adenoviruses induce oncogenic transformation of mammalian cells.

APPROACH: Two main avenues are being pursued: Firstly, the properties of transformation defective host range (hr) mutants of adenovirus type 5 (Ad 5) are being studied in order to identify the viral products involved in cell transformation and to elucidate the role of these products in transformation as well as in viral replication. Early polypeptides from wt or hr mutant infected HeLa cells and from Ad 5 transformed cells are being analyzed by immunoprecipitation and SDS-polyacrylamide electrophoresis. Immunoprecipitated polypeptides are being further characterized by proteolytic cleavage and peptide mapping. An attempt is being made to detect deletions or base substitutions in mutant viral DNA by forming hr-wt heteroduplexes, cutting regions of mismatch with S1 nuclease and following specific cleavage with various restriction enzymes, analyzing the products by agarose gel electrophoresis. Base sequence alterations in mutant DNA will then be compared with any polypeptide changes which might be detected by peptide mapping.

The second approach being followed involves establishing adenovirus transformed cell lines (transformed by various restriction enzyme fragments or Ad 5 of Ad 12 DNA, or by mutants carrying altered transforming genes and correlating oncogenicity of these lines with other cellular properties.

PROGRESS: A number of hr mutants have been isolated and partially characterized. The mutants fall into two complementation groups one of which is transformation defective, DNA positive, and fails to induce the synthesis of the 58K tumor antigen of Ad 5. Mutants of the other complementation group are DNA negative, induce an abnormal transformation of rat kidney cells, and are defective in the synthesis of a 10.5 K polypeptide.

Studies on tumorigenicity of adeno transformed cells indicate that rat cells transformed by the EcoRI C fragment of Ad 12 do not differ from virus transformed lines in terms of tumorigenicity. Lines transformed by hr Ad 5 mutants, however, may have a lower tumorigenicity than cells similarly transformed by wt Ad 5.

360. TRANSFORMATION AND GENE REGULATION BY ADENOVIRUSES

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This project undertakes an analysis of the mechanisms of transformation and gene regulation by human adenoviruses. Deletion mutants of adenovirus type 2 (Ad 2), isolated from laboratory stocks, or constructed in vitro, will be used to identify regions of the viral genome involved in transformation or the regulation of gene expression. The phenotypes of mutants with apparent defects in transformation or regulation will be carefully studied to determine how the deleted regions of the genome are involved in these processes.

361. TRANSFORMING PROTEINS OF THREE HUMAN ADENOVIRUS GROUPS

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We have recently shown by immunoprecipitation, peptide mapping, and in vitro translation that E1, the transforming gene region of the human adenovirus 2 (Ad2) genome, encodes two or more 'families' of partially related polypeptides. One family, coded by E1a (mp 1.5-4.5) includes four 40K-50K polypeptides that are partially related; a second family coded by E1b (mp 4.5-11), includes major 53K and 15K polypeptides that are highly related. Minor polypeptides of 14K-16K and 18K-20K were detected that are part of the 53K/15K family. Polypeptides of 28K and 11K-12K were also immunoprecipitated that are unrelated by peptide mapping to each other or to E1a-40K-50K or to E1b-53K/15K. In this proposal, we will (1) investigate whether the 28K and 11K-12K are E1-coded, by peptide mapping of polypeptides translated in vitro, (2) determine whether the E1-coded polypeptides are synthesized in 6 lines (F17, T2C4, 8617, F4, FRK, 293) of Ad-transformed cells, using radioimmunoprecipitation, O'Farrell 2D gels, and peptide mapping, (3) investigate the polypeptides specified by transformation-defective host range mutants with deletions in E1, in order to identify and map E1-coded polypeptides, and to relate specific polypeptides to the phenotypes of these mutants, (4) purify the E1b-53K and 15K polypeptides to homogeneity, attempt to purify E1a-40K-50Ks, and perhaps the minor E1-polypeptides, prepare monospecific antisera, and attempt experiments to illuminate the functions of these polypeptides, (5) sequence the N-termini of the E1b-53K, E1b-15K, E1a-40-K-50Ks, and partially sequence the N-termini of cyanogen bromide fragments in order to align the structural genes for these polypeptides with the viral DNA sequence. We will also do limited analyses of E1-coded polypeptides of group A (Ad12), group B (Ad12), and group B (Ad7) Ads, for comparative purposes with group C (Ad2, 5) Ads. Our studies may contribute to our understanding of the functions of the E1-coded polypeptides in cell transformation and virus replication, and to the organization and regulation of overlapping split genes.

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362. CELL TRANSFORMATION BY DEFECTIVE VIRUS PARTICLES

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Transformation of mammalian cells is a useful model system for viral oncogenesis. Little is known about what factors govern the difference in response of different cell (species) types to viruses; whether the cell will be killed or transformed, whether virus will replicate its DNA, be transcribed, have viral DNA integrated into cell DNA or completely degraded. Various serotypes of human adenoviruses differ in their interactions with given cell types at the level of DNA replication, and specifically with the amount of defective genomes which are generated. I am studying the synthesis of viral DNA, complete and defective, in a variety of combinations of cell types and virus serotypes and the eventual transformation of some cells by virus. The goals of this research are to understand the nature of the primary events which determine whether or not a cell will be transformed, and to test the hypothesis that defective genomes play a specific role in transformation. I am emphasizing a systematic study of virus-cell interactions which have been studied haphazardly, heretofore, by different researchers using techniques different enough to make comparison difficult.

BIBLIOGRAPHIC REFERENCES: Daniell, E. and T. Mullenbach. Synthesis of Defective Viral DNA in HeLa Cells Infected with Adenovirus Type 3. J. Virol., in press (April, 1978). Kit, G. and E. Daniell. Adenovirus Core Protein Synthesis in the Absence of Viral DNA Synthesis Late in Infection. J. Virol., in press (July, 1978).

363. COTRANSCRIPTS OF VIRAL AND HOST DNA IN CELLS PRODUCTIVELY INFECTED OR TRANSFORMED BY AN ONCOGENIC VIRUS

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The summary for this project is under entry no. 328.

364. VIRAL GENE EXPRESSION AND ITS REGULATION IN PRODUCTIVELY INFECTED AND TRANSFORMED CELLS

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The summary for this project is under entry no. 340.

365. KARYOTYPIC AND PHENOTYPIC CHANGES IN THE PROGRESSION OF VIRALLY TRANSFORMED CELL LINES (ABBREV)

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The summary for this project is under entry no. 215.

366. VIRAL ONCOGENESIS BY DNA VIRUSES

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OBJECTIVE: To study adenovirus genes and their functions involved in transformation and tumorigenesis.

APPROACH: Adenovirus type 12 is highly oncogenic. We want to study what viral gene products are responsible for oncogenicity. We also want to study the defects in a class of Ad 12 mutants (cyt mutants) which have low oncogenicity. Primary rat kidney cells will be used for transformation studies. Clones of transformed cells resulting from these studies will be analyzed for viral gene content and their expression using conventional DNA reassociation, DNA-RNA hybridization techniques. Viral DNA fragments generated by restriction endonucleases will be used as reagents for hybridization and for cell transformation in vitro.

PROGRESS: We have obtained clones of rat kidney cells containing about 10% of the left hand end of Ad 12. These cells are oncogenic in immunocompetent rats. Sera from animals bearing tumors induced by these transformed cells can immunoprecipitate polypeptides having molecular weights of 42-46K, 16.5K and 10.5K from Ad 12 infected KB cells. We have obtained data that cyt mutants transform rat kidney cells at a very low frequency compared to wild type Ad 12. However, cells transformed by the cyt mutants are also highly oncogenic in immunocompetent animals. These data suggest that the defect(s) in cyt mutants may be in the initiation of transformation. In these transformed cells, viral RNA coded by the left hand end of the Ad 12 genome is synthesized.

367. ADENOVIRUS T, SURFACE ANTIGENS AND TUMORIGENICITY

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Human adenoviruses have well-documented oncogenic potential in experimental animals and in vitro. Adenoviruses are not viewed as candidates for a major etiological role in human malignancy but they serve as a convenient probe in tumor biology. The transforming capacity of adenoviruses is associated with the left hand end of their genome which codes for transformation proteins. The products of this region of adenovirus type 12 genome will be isolated and characterized. We will attempt to define the mechanisms by which these products establish oncogenic transformation. Although all adenoviruses can transform cells in vitro, in vivo oncogenic potential of different serotypes varies from highly oncogenic to nononcogenic. The adenovirus-specific surface antigens will be identified in transformed cells and their role in the induction of the immune response will be analyzed. The immune response to cells transformed with highly oncogenic and nononcogenic adenoviruses will be qualitatively and quantitatively compared in syngeneic hosts. Data will be obtained indicating whether the immune response plays a major role in differences in tumorigenicity of individual adenoviruses in vivo.

BIBLIOGRAPHIC REFERENCES: Geis, A., Fohring, B. and Raska, K., Jr. VA RNA synthesis in adenovirus type 12 infections. Federation Proceedings 38, 1071 (1979). Fohring, B., Geis, A., Koomey, M. and Raska, K., Jr. Adenovirus type 12 VA RNA. I. Synthesis in productive infection and gene mapping. Virology, in press (1979).

368. NOVEL METHODS TO MAP VIRAL FUNCTIONS

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The summary for this project is under entry no. 237.

369. ISOLATION AND CHARACTERIZATION OF HOST-RANGE MUTANTS OF HERPES SIMPLEX VIRUS TYPE II AND ADENOVIRUS

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The summary for this project is under entry no. 45.

370. STUDIES OF VIRAL ANTIGENS IN VIRUS-INDUCED TUMORS

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The initial objectives of this project were to use human adenoviruses and adenovirus-SV40(Ad2-240) recombinants as tools to study the genetics of DNA tumor viruses, to define the role of viral genes and viral antigens in viral oncogenesis and to study the biology of Ad2-SV40 recombinants. Due to the lack of proper containment facilities in NIAID between January 1973 and May 1978, there has been a 5-year disruption (see past reports) in the major thrust of this project. During this interval, a study of Ad2-SV40 recombinant-associated tumor induction by these agents with the incorporation into the adenovirus 2 chromosome of a specific segment of SV40 DNA. To begin to understand the mechanism by which this SV40 DNA segment conveys oncogenicity to these recombinants, it was necessary to understand why Ad2 was nononcogenic for hamsters. Since Ad2 will transform hamster cells in tissue culture, we initiated a study of the oncogenic properties of Ad2 transformed cells. The results thus far lead us to suspect that unrecognized host immune mechanisms in the hamster reject Ad2 transformed cells and transformed cell-induced tumors. Such a concept suggests that the presence of a specific segment of the SV40 genome might induce functions in hamster tumor cells that interfere with the rejection process. Future studies will be directed toward more carefully defining these concepts.

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371. A PROGRAM IN VIRAL ONCOLOGY

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The summary for this project is under entry no. 42.

IV. OTHER DNA TUMOR VIRUSES

A. STRUCTURE AND GENOMIC ORGANIZATION

372. STRUCTURE AND FUNCTION OF DNA VIRUSES IN RELATION TO TUMOR FORMATION

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OBJECTIVE: Analyze the structure of the chromatin of small oncogenic DNA viruses. Study its interactions with the chromatin of the host cells.

APPROACH: Both reconstituted and natural viral chromatins are analyzed and the movement of the nucleosomal proteins along the DNA is being studied. The influence of transcription on the relative movement of histones to DNA is being analyzed. These studies include the action of both endogenous RNA polymerase and E. coli RNA polymerase. The role of polymerase in the folding of the DNA is being investigated.

PROGRESS: Nucleosomes can move along the DNA even in the absence of RNA polymerase. These data have been

published (Cell 15, 955, 1978). A new finding is that E. coli RNA polymerase can efficiently use SV40 chromatin as a template.

373. STUDIES ON NUCLEIC ACIDS

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OBJECTIVE: Determination of the sequence of parvovirus DNA.

374. BOVINE PAPILLOMAVIRUS DNA AND NEOPLASIA

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Bovine papillomavirus (BPV) type 1 and type 2 DNA sequences have been detected in numerous naturally occurring equine connective tissue tumors and cell lines established from virus-induced equine and bovine fibromas. Most of the sequences appear as superhelical forms with no evidence of integration into the host genome. To delineate the function of the BPV genome in these tumor cells, virus-specific RNA transcripts will be positioned on the restriction endonuclease cleavage maps of the virus genomes. Mouse cells transformed by BPV and other papillomaviruses will be analyzed for integrated viral DNA sequences and synthesis of virus mRNA.

Conserved polynucleotide sequences have been shown to exist among the genomes of papillomaviruses of human, rabbit and bovine origin. Studies are under way to further examine the extent of this evolutionary relatedness among these viruses. To obtain sufficient quantities of virus DNA for study, DNAs from human, dog, ox, horse, goat and deer papillomaviruses are being molecularly cloned. Additionally, various tumors are being examined for conserved papillomavirus polynucleotide sequences employing intact virus DNA and subgenomic fragments as probes.

BIBLIOGRAPHIC REFERENCES: Law, M.-F., W.D. Lancaster and P.M. Howley. 1979. Conserved polynucleotide sequences among the genomes of papillomaviruses. J. Virol. 32:199-207. Lancaster, W.D. 1979. Physical maps of bovine papillomavirus type 1 and type 2 genomes. J. Virol. 32:684-687.

375. ANALYSIS OF THE SHOPE PAPILLOMA-CARCINOMA SYSTEM

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The Shope papilloma-carcinoma system has great potential for the definition of basic biochemical phenomena which are critical to the neoplastic process. This is evident since in domestic and cottontail rabbits there is a virally induced progression from normal skin to papilloma to carcinoma. We have shown that multiple copies of the viral genome are conserved in all neoplasms and that some 5% of viral genetic information is transcribed in tumors of domestic rabbits (which characteristically do not produce virus). That multiple copies of the virus genome are present in viral superinfected chemically induced papillomas and in serially transplantable VX-2 and VX-7 carcinomas has also been shown.

In the present application we propose to determine: 1) Whether the same transcripts are present in all tumors. 2) Whether or not the viral DNA is covalently integrated into the host cell genome. 3) If there is a translation product in these tumors and if so, its basic properties (since it is likely to be involved in an induction and maintenance of the neoplastic state). 4) The viral specific macromolecular events which accompany malignant change induced by the virus in superinfected papillomas established by a chemical carcinogen. In addition, in related studies the effect of a tumor promotor on the expression of viral genes will be assessed. 5) If VX-2 and VX-7 carcinomas resemble the primary tumors with respect to viral gene expression. Should this prove to be the case, cultures of these cells will be intensively studied to determine the nature of the virus cellular relationship as it relates to these neoplastic cells. 6) If using additional methods, cell lines from the virus tumors can be established in vitro. Molecular hybridization, restriction endonuclease digestion, immunoprecipitation of radioactive materials and cell culture methods appropriate to epidermal cells will be the primary tools employed in these studies.

BIBLIOGRAPHIC REFERENCES: Stevens, J.G. and Wettstein, F.O. Multiple Copies of Shope Virus DNA are Present in Cells in Benign and Malignant Non-Virus-Producing Neoplasms. J. Virol. 30, 891 (1979). In Press (1980). Wettstein, F.O. and Stevens, J.G. Distribution and State of Viral Nucleic Acid in Shope-Papilloma Virus Induced Tumors. In: Viruses in Naturally Occurring Cancers (M. Essex, G. Todaro, and H. Zur-Hausen, eds). Cold Spring Harbor Laboratory.

376. DETECTION OF HUMAN WART VIRAL DNA IN MALIGNANT TISSUE

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The bovine papilloma viruses appear to be the most oncogenic of the papilloma viruses. In order to study some of the relationships occurring between this virus and its host, BPV transformed cell lines will be established and subsequently characterized. Specifically, we will examine transformed cell lines for viral and virally induced antigens, BPV DNA sequences and BPV-specific mRNA. In addition, we will construct a physical map of BPV DNA and relate it to function.

377. STRUCTURE OF HERPESVIRUS GENOMES

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The summary for this project is under entry no. 61.

378. GENETIC EXPRESSION OF DNA TUMOR VIRUSES

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This is part of a broader project. A summary of this subproject is not available.

B. VIRUS REPLICATION

379. TRANSCRIPTION AND TRANSLATION IN POX VIRUS INFECTED CELLS

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The role of poxviruses, subviral particles isolated from virions or purified poxviral DNA in causing abnormal proliferation or neoplastic transformation of human and murine cells will be investigated. The structure and replication of ortho and parapoxvaccinia DNAs will be studied in order to define: (a) The nature of the viral chromosomal structure and (b) the viral 'replisome', in order to gain insight into the structure and replication of intrinsically more complex eukaryotic cell chromosomes. Alterations in cellular membranes following infection with various vaccinia strains will be compared and defined to: (a) Determine how such alterations affect cell growth and cell-cell recognition; (b) function in providing 'targets' recognized by the host's immune system. Restriction enzyme analysis and molecular hybridization techniques will be used to probe the genetic relatedness of poxviruses and their diversification in nature. This will provide the basis for future studies of the genetic information needed for determining host range specificity, pathogenicity and cell proliferation or killing after infection of cells with poxviruses.

BIBLIOGRAPHIC REFERENCES: Esteban, M., and Holowczak J.A. Vaccinia Virus DNA Replication. American Society for Microbiology: Microbiology 1980 (In Press). Soloski, M.J., and Holowczak, J.A. Studies concerning the structure and organization of the vaccinia virus nucleoid. I. Isolation and characterization of subviral particles prepared by treating virions with Guanidine-HCl, Nonidet P40 and 2-mercaptoethanol. Virology 99: 209-217 (1979).

380. NUCLEAR INVOLVEMENT IN THE REPLICATION OF VACCINIA DNA

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The aims of this research program are to study the replication of vaccinia virus DNA and to use vaccinia as a probe and a model system in understanding the control mechanisms involved in regulating DNA replication in normal cells. We propose to investigate the possible nuclear involvement in the DNA replication of vaccinia virus, a virus which has until recently been thought to replicate in the cytoplasm without any dependence on the cell nucleus. We will first determine whether the viral DNA is present and replicating in the nucleus. Different techniques for fractionation of infected, synchronized cells, including the method of cytochalasin induced enucleation, will be employed in preliminary studies. Differential labeling and density gradient analyses will be used to provide a biochemical picture of the replication process in both locations in isolated cytoplasts and karyoplasts. We plan to transplant infected karyoplasts into uninfected cytoplasts, which should provide a unique method for studying possible precursor/product relationships. Transplantation studies will also be used to answer questions concerning the possibility of other types of nuclear involvement in viral DNA replication. Regardless of the site of replication or existence of nuclear involvement, we are interested in examining the processes of vaccinia DNA replication by autoradiographic and other techniques. Because of the large size of vaccinia DNA, it will be possible to use DNA fiber autoradiographic studies to provide a dynamic picture of replication and to discover the number and location of the origins of replication, and the rate and direction of replication.

381. THE REPLICATION OF PARVOVIRUS DNA

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The chromosome of the parvovirus MVM is a linear, nonpermuted, single-stranded DNA molecule with duplex hairpin structures at both termini. The hairpin at the 3' (left-hand) end acts as a primer, in vitro, for a number of DNA polymerases including that of the bacteriophage T4. Highly purified T4 DNA polymerase, like most other polymerases, will catalyze the synthesis of the complementary strand, and stops on reaching the 5' end of the hairpin at the right-hand terminus. In concert with the T4 gene 32 protein, gene 45 protein and the gene 44-62 protein complex, T4 DNA polymerase can strand-displace this hairpin, producing a palindromic terminus. With low efficiency this reaction also yields larger products of the molecular weight expected for dimer forms of duplex viral DNA. We are using this reaction to prepare pure palindromic termini with which we will determine the sequence arrangements of the DNA within the right-hand terminal hairpin, and to investigate the role of this structure in the replication of the viral DNA in vivo.

382. REPLICATION OF THE PARVOVIRUS KRV

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We have been studying the specific biochemical mechanisms involved in the replication of the autonomous parvovirus KRV in a rat nephroma cell line. The virus, isolated originally from a rat sarcoma, contains one molecule of linear, single-stranded DNA and three capsid proteins. Little information is available about replication of a single-stranded DNA virus in a eukaryotic cell or on the transcription of this DNA to make viral proteins. We have demonstrated that the virion KRV-DNA can self-prime in vitro to synthesize the double-stranded replicative intermediate. In an attempt to resolve this self-priming terminus, so important in DNA replication, we have sequenced the nucleotides in the 3' DNA terminus which probably also contains the in vitro origin of DNA replication.

BIBLIOGRAPHIC REFERENCES: Salzman, L.A., P. Fabisch. Nucleotide sequence of the self-priming 3'terminus of the single-stranded DNA extracted from the Parvovirus, Kilham Rat Virus. J. Virol. 30: 946-951 (1979). Wali, T.M. and Salzman, L.A.,. Sedimentation analysis of the RNA synthesized in vivo by the autonomous parvovirus, Kilham Rat Virus. J. Virol. 1979 (in press).

383. PARVOVIRUS VARIANTS SPECIFIC FOR HOST CELLS WITH DISSIMILAR DIFFERENTIATED PHENOTYPES

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The genome of the parvovirus MVM is believed to comprise a single transcription unit, coding for the three coat proteins, which have overlapping amino acid sequence. In addition to the coding sequence we assume that the genome expresses whatever secondary structures and 'signal' sequences are required to direct the replication, transcription and packaging of the viral DNA. Because these processes are almost certainly carried out by host proteins we would predict that such viral control regions mimic similar sequences employed by the host, allowing the virus to suborn cellular machinery for its own use. It has been shown that MVM strain T requires a differentiation-induced replication function, dirf, expressed constitutively in only a limited number of differentiated cell types, for instance in fibroblasts but not in lymphocytes. The discovery of a variant of MVM, strain B, which grows in lymphocytes, but not in fibroblasts, suggests that there may be a number of dirf genes, expressed in different cell types and exploited by different virus variants. The use of somatic cell hybrids and infectivity assays which distinguish between the two viruses has shown that each dirf gene product probably interacts with a variant specific sequence within the appropriate viral genome, to effect an essential step in replication. We have used restriction endonuclease mapping and heteroduplex analysis to look for differences at the nucleotide sequence level between the T and B strains of MVM. At present the major difference, which includes a 50-100 nucleotide sequence present in T but not in B, has been mapped at the extreme right-hand end of the molecule, close to the 5' hairpin of the viral strand.

384. DIFFERENTIATION-CONTROLLED HOST GENES INVOLVED IN PARVOVIRUS REPLICATION

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Non-defective parvoviruses are dependent, for lytic growth, upon host function(s) expressed transiently during the cell cycle, explaining the predilection for rapidly proliferating tissues that these viruses exhibit in vivo. Infection of teratocarcinoma stem cells and their differentiated derivaties has shown that cell cycling is a necessary, but not sufficient, condition for lytic virus growth, and that the host cells' differentiated state is also important. The infection of stable fusion hybrids and heterokaryons formed between stem cells and permissive derivatives, and between permissive and non-permissive differentiated cell types, has demonstrated that virus replication is dependent upon a host gene(s) which is expressed in only a small number of differentiated cell types. This differentiation-induced replication function, DIRF, is required for virus replication but not for cell growth, is not extinguished in somatic cell hybrids and acts in the virus life cycle at a stage after penetration. We have isolated a number of virus-resistant cell clones from sensitive cell populations and have partially characterized them. They arise at high frequency and appear to be epigenetic variants which are relatively stable in culture. Cell fusion has shown that some of these clones are apparently suppressed for the expression of DIRF. We hope that further analysis of these cell lines will enable us to find out how many DIRF genes there are, how they act in virus replication and how their expression is epigenetically controlled.

385. EVENTS OF PARVOVIRUS AND ANIMAL CELL DNA SYNTHESIS

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We propose to use the parvovirus MVM as a means of investigating the events of eukaryotic DNA synthesis as they occur in vivo. The replication of MVM DNA is known to be dependent upon events which occur only in late S phase of the host cell. The extremely small size of the viral genome and consequent dependence on host cell functions make MVM an ideal tool for the study of host mechanisms that are specific to a portion of the cell cycle. Our objective is to determine which of

the steps in viral DNA replication are specifically coupled to the host cell cycle. Two specific areas will be studied. First, we wish to determine if viral DNA integration into the host cell DNA serves as a means of coupling host and virus DNA synthesis. We also propose to determine when in the host cell cycle the viral single-stranded DNA is first converted to its double-stranded replicating form. These experiments involve the isolation of various forms of the replicating viral DNA from highly synchronized, infected cells.

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386. REPLICON CONTROL IN NORMAL AND TRANSFORMING CELLS

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The summary for this project is under entry no. 181.

387. REGULATION OF PARVOVIRUS GENE EXPRESSION

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The general objectives of this investigation are: first, to describe the viral transcription program during parvovirus H1 replication in rodent cells; second, to elucidate the components and mechanisms involved in the activation (transcription) of the viral genome; and, ultimately, to apply what is learned from the analysis of this presumably simple transcription system to other genetic systems with more complicated transcription programs and regulatory mechanisms. To this end the specific aims are (1) to characterize the intracellular viral DNA forms, one of which must serve as the template for transcription, (2) to map on the viral genome both stable transcripts (mRNAs) and nascent nuclear transcripts in order to identify transcription initiation and termination sites, as well as processing events, involved in the generation of functional mRNAs, (3) to isolate and characterize RNA polymerases and associated components from host cells, (4) to reconstruct specific transcription events with nuclear templates, purified RNA polymerases, and other soluble components, (5) to isolate and characterize viral nucleoprotein templates or transcription complexes which are functional in reconstructed systems, (6) to reconstruct faithful transcription systems from purified DNA templates, purified RNA polymerases, and other soluble or template-associated components, and (7) given the identification of the transcription components via these studies, to investigate the sites of interaction and mechanism of action of these components. In all these studies the functions of specific components will be inferred on the basis of their abilities to effect transcription events in vitro which are identical to those observed in intact cells.

388. STUDIES ON DNA SYNTHESIS

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We propose to study DNA synthesis of parvovirus H-1 as a model for DNA synthesis in animal cells. The studies will include (1) purification of a soluble system from uninfected cells that converts H-1 viral single strands to replicative form (RF) DNA; (2) characterization of the protein covalently bound to the 5' termini of H-1 RF DNA; (3) analysis of the structures of replicative intermediates; and (4) development of a soluble system for in vitro replication of double-stranded RF DNA.

Second, we propose to study the ability of the mechanism for synthesis of the RNA primer for animal cell DNA synthesis to accept deoxynucleotides, nucleotide analogues and noncomplementary bases. The studies will be carried out both in vitro and in vivo and will include an assessment of possible effects of the misincorporation on DNA synthesis.

Third, we propose to study the consequences of expansion of intracellular deoxyuridylate pools and contraction of thymidylate that result from treatment of cells with FdUrd. The studies will include (1) measurement of the intracellular pools for dUTP, FdUTP and dTTP; (2) measurement of the misincorporation of dUMP and FdUMP into DNA; and (3) analysis of the relationship between misincorporation/removal of Ura and FUra, DNA fragmentation, and cell death.

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389. STRUCTURAL BASIS FOR REGULATION OF DNA VIRUS GENE EXPRESSION

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The summary for this project is under entry no. 59.

C. VIRUS-HOST INTERACTIONS

390. CONTROL OF GENE EXPRESSION IN TUMOR VIRUSES AND CELLS

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The summary for this project is under entry no. 141.

391. HEPATITIS B VIRUS AND HEPATOCELLULAR CARCINOMA

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A substantial amount of information linking the hepatitis B virus and hepatocellular carcinoma has been generated along epidemiological and serological lines. The objectives of the research proposed here are to obtain data to more strongly link the virus in the direct production of hepatocellular carcinoma.

Using the techniques of in vitro and in situ nucleic acid hybridization, homologous DNA will be sought to the hepatitis B Dane particle DNA in hepatitis B associated and nonassociated chronic liver disease and malignancy. Attempts will be made to directly localize the homologous DNA to the cell genome by 1) in situ hybridization to metaphase chromosomal spreads from human hepatocellular carcinoma tissue culture line and 2) further evaluation of high molecular weight DNA from chronically infected liver which is hybridizable to viral DNA probe.

In addition, to further understand the events leading from acute infection to chronic infection to malignant transformation, a newly described animal model involving Marmota monax, the common woodchuck, will be investigated for its applicability to the hepatitis B setting. Also, human hepatoma tissue cell lines will be transplanted into nude athymic mice to further observe anti-viral, anti-tumor and immunologic manipulation of the malignant cells.

BIBLIOGRAPHIC REFERENCES: Lutwick, L.I.: Relationship between aflatoxin, hepatitis B virus and hepatocellular carcinoma. Lancet i-755-757, 1979.

392. MOLECULAR BASIS OF GENETIC RECOMBINATION (BACTERIA, VIRUSES)

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The summary for this project is under entry no. 65.

D. CELLULAR TRANSFORMATION

393. RADIATION AND CHEMICAL EFFECTS ON VIRAL TRANSFORMATION AND TUMOR ANTIGEN EXPRESSION

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The objective of this research is to define the role of low level irradiation combined with DNA containing viruses known to exist in the human population to determine the potentiating effects of irradiation on virally induced neoplasms. The second aspect of the work is to define the role of low levels of chemical carcinogens, particularly hydrocarbons, in potentiating the oncogenic properties of viruses. The primary focus of a large

portion of the work will center around determining whether or not it is possible to detect the earliest signs of virally or chemically induced tumors, potentiated by hydrocarbons or irradiation through the monitoring of cells or the immune response of the host to tumor associated antigens which appear to be of embryonal or fetal origin. The approach being used is to detect the earliest transformed cells that appear in cultures of cells exposed to cocarcinogens as reflected by their ability to grow on an unusual test culture surface, polyacrylamide, which seems to have the unique property of excluding the growth of normal cells while potentiating the growth of cancer cells. Cells which become adherent to the acrylamide will be monitored for the expression of embryonic antigens which are common to tumors of the same histologic type. The results expected should allow us to determine whether embryonal or fetal immunologic markers can be used to detect the earliest evidence of chemically or virally induced carcinogenesis which can be potentiated by low level irradiation. We anticipate that our results will be in hand in the coming three years.

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394. ROLE OF LATENT VIRUSES IN RESISTANCE

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It has been determined by us and others that several of the parvoviruses interfere with viral oncogenesis both in vivo and in vitro. The possible role of these ubiquitous agents in cancer resistance has encouraged us to study the phenomenon in detail. We hope to define the spectrum of interference between certain parvoviruses, in particular H-1 virus originally isolated from human tissue, and various oncogenic agents (viral and chemical) which may prove relevant to human malignancy. The biological interplay between H-1 virus and these transforming agents, some of which act as 'helpers,' will be explored in productive and non-productive host cell infections. The molecular basis of the interference with malignant transformation, and 'helper' virus replication will be sought through an understanding of the molecular biology of H-1 replication. Our growing bank of conditional lethal mutants of H-1 virus will be an important tool for this.

The simplicity of H-1 virus and its biochemical dependence on host cell replicative machinery has proved to be more specific than at first realized; this makes the virus a valuable tool for probing many aspects of host cell function.

An important aspect of parvovirus biology appears to be latency, and tissue culture and animal models for this will continue to be examined for the mechanism of this phenomenon.

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395. MALIGNANT TRANSFORMATION OF ANIMAL CELLS BY DNA TUMOR VIRUSES

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This is part of a broader project. A summary of this subproject is not available.

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